Development of a Bioassay Protocol for Evaluating the Toxic Risk to Regional Fisheries Resources Posed by Forest-use Herbicides

Written and Prepared by:
D. McLeay

D. McLeay & Associates Ltd.
300 - 1497 Marine Drive
West Vancouver, B.C. V7T 1B8

October 1988

ECONOMIC & REGIONAL DEVELOPMENT AGREEMENT

Canada

BC

Funding for this publication was provided by the Canada-British Columbia Forest Resource Development Agreement - a five year (1985-90) $300 million program cost-shared equally by the federal and provincial governments.

Canadian Cataloguing in Publication Data

McLeay, D. J. (Donald James), 1943–
Development of a bioassay protocol for evaluating the toxic risk to regional fisheries resources posed by forest-use herbicides

(FRDA report, ISSN 0835–0752 ; 039)
Issued under Forest Resource Development Agreement.
Co-published by B.C. Ministry of Forests.
On cover: Canada/BC Economic & Regional Development Agreement.
Bibliography: p.
ISBN 0–7726–0859–8

1. Herbicides – Toxicology – Measurement. 2. Water – Pollution – Toxicology – Measurement. 3. Salmonidae. 4. Freshwater invertebrates. 5. Water quality bioassay. I. Canadian Forestry Service. II. British Columbia. Ministry of Forests. III. Forest Resource Development Agreement (Canada). IV. Canada/BC Economic & Regional Development Agreement. V. Title. VI. Series.

SB951.4.M34 1988 632.954 C88–092184–6

© 1988 Government of Canada,
Province of British Columbia

This is a joint publication of the Canadian Forestry Service and the British Columbia Ministry of Forests.

Produced and distributed by the Ministry of Forests, Research Branch.

For additional copies and/or further information about the Canada-British Columbia Forest Resource Development Agreement, contact:

Canadian Forestry Service
Pacific Forestry Centre
506 West Burnside Road
Victoria, B.C. V8Z 1M5
(604) 388-0600

or

B.C. Ministry of Forests
Research Branch
31 Bastion Square
Victoria, B.C. V8W 3E7
(604) 387-6719

EXECUTIVE SUMMARY

Presently, no standardized approach exists for assessing the immediate or long-term toxic threat to anadromous and resident salmonid fish resources, posed by existing or candidate forestry herbicides. The types of aquatic toxicity tests currently being used are varied and provide no consistent means for comparing individual herbicides for relative toxicity. Nor do they necessarily relate to the freshwater life of primary concern (Pacific salmon and trout and their prey), or to the nature of exposure these species encounter when herbicides are applied within British Columbia forests.

This report reviews the bioassay test procedures used to date for evaluating the toxicity of herbicides to salmonid fish and sensitive freshwater invertebrate species. Based on this review, and in consideration of regional needs as well as the most promising and applicable test procedures and approaches, a tentative bioassay protocol is developed and presented.

Literature Review

Acute lethal tests

The median lethal concentration (LC50) test is the benchmark bioassay for measuring the acute toxicity of herbicides and other aquatic contaminants. The acute (i.e., within 4 days or less) lethal toxicity of herbicides to salmonid fish and sensitive freshwater invertebrate species has, in most instances, been measured under controlled static (no solution replacement) conditions. Some researchers have used the more expensive static replacement, or flow-through, procedures to ensure sample stability during testing. Few tests, however, have compared the effect of solution replacement on toxicity under otherwise identical conditions.

Young (swimup fry or fingerling) rainbow trout are the salmonid fish species most commonly used for LC50 tests. Hatchery-reared coho and other Pacific salmon species have also been employed without problems. Of the various fish life stages tested, alevins (yolk-sac fry) and young free-swimming fish have often been found to be more sensitive to herbicides and other aquatic contaminants than are eggs or older (juvenile, adult) life stages. Most fish LC50 tests are continued for 96 h, during which time their percent survival (usually for groups of 10 fish exposed to a range of herbicide strengths) is monitored daily.

Daphnia magna is the invertebrate species used most commonly for testing the toxicity of herbicides to freshwater fishfood organisms. As with LC50 tests with salmonid fish species, standardized procedures that describe "accepted" methodologies for culturing this organism and for testing its acute tolerance to contaminants are now available. A number of laboratories throughout Canada are experienced with LC50 tests using daphnids. These bioassays are normally conducted as static 48-h LC50 tests. Other freshwater fishfood organisms, including amphipods (*Gammarus sp.*), larvae of *Chironomus sp.*, and mayfly (e.g., *Hexagenia* and *Ephemerella sp.*) or stonefly (*Pteronarcys sp.*) nymphs, are useful and sensitive test organisms. However, their use in toxicity tests with herbicides has been limited, and test procedures using these species have yet to be "standardized."

Acute sublethal tests

Few studies have tried to determine the threshold concentrations at which herbicides cause sublethal responses when exposures are brief (hours, few days).

Behavioral tests have measured median effective concentrations (EC50) of certain herbicides (e.g., Krenite®, Roundup®) that cause avoidance responses for young salmonid fish within 1 h. The occasional study has attempted to measure the lowest sublethal concentrations of herbicides that may cause acute stress responses in salmonid fish. Salinity challenge tests have also been used to determine if prior exposure of salmon smolts to sublethal strengths of herbicides reduces their ability to survive and osmoregulate when transferred to seawater. However, little effort has been made to quantify threshold sublethal concentrations of herbicides harmful to freshwater invertebrate species, during or following their short-term exposure.

Chronic sublethal tests

The majority of controlled tests for long-term effects of herbicides on salmonid fish have been partial life cycle studies, in which groups of eggs or alevins are reared for a minimum of 60 days while exposed to a range of dilute herbicide strengths. Test solutions are, in most instances, renewed throughout the tests. Effects on egg and alevin survival and development, and on the growth and survival of fry and fingerlings, are monitored and related to herbicide concentration. A second approach has been to determine how brief (few days) exposure of early life stages of Pacific salmon and trout to sublethal herbicide strengths, affect their subsequent long-term survival, development, and growth in freshwater. The occasional study has examined fish for histological changes during prolonged herbicide exposure. However, little effort has been made to quantify fish behavioral, physiological, or biochemical responses to chronic herbicide exposure, or to measure delayed effects on such responses following brief exposure.

A number of herbicides have been tested for their chronic toxic effects on *Daphnia magna* or *Daphnia pulex*, using controlled life cycle tests. These tests determine the Lowest Observed Effect Concentration (LOEC) that will cause significant impairment of reproduction, growth, or long-term survival, and can be completed in 21 days.

Effect of environmental variables

A few acute lethal toxicity studies using salmonid fish have tried to determine the extent to which certain environmental variables may modify herbicide toxicity. Depending on fish species and herbicide tested, temperature variations of 10°C or less caused as much as a 5-fold difference in 96-h LC50 values. Changes in pH within the range 6.5-8.5 resulted in as much as a 40-fold difference in toxicity. Depending on the herbicide, fish LC50 values may be increased, decreased, or unaffected by lowering test pH within this range. The effect of aging dilute herbicide solutions on fish LC50 values has also been studied. For certain herbicides, including Roundup®, picloram, and Krenite® , little if any change in toxicity was found when aqueous solutions were aged for up to 28 days under bioassay test conditions. One study demonstrated that a variety of natural, uncontaminated freshwater samples, when each was used as the diluent water in otherwise identical acute lethal tests with rainbow trout and the same herbicide, caused a 12-fold variation in LC50 values. The pH of these natural freshwater samples varied from 6.7 to 8.2.

Field toxicity tests

Few reports describe field evaluations of the toxic effects of forestry herbicides on salmonid fish or freshwater invertebrate species. Studies with caged fingerling salmon and trout, held in forest streams receiving direct overhead spraying, spray drift, or surface runoff from treated areas, have been useful in demonstrating fish survival and the possible sublethal toxic effects on fish behavior during and after spraying operations. One report described the downstream migration of coho salmon smolts previously held in sublethal concentrations of a number of herbicides for periods of 4-15 days. Of the three herbicides examined, one (diquat) caused effects at strengths as low as 0.02 of the 96-h LC50 value; no effects were caused by the other herbicides at any sublethal strength tested. Another study reported the successful use of caged *Daphnia magna*, observed for survival rates up to 8 days following the intentional manual spraying of a forest pond with Roundup®. No reports of caging studies with other freshwater invertebrate species were found in the literature, although a number of stream invertebrate species including mayflies and stoneflies proved a worthwhile predictor of the impact of an aerial insecticide application on stream invertebrate drift. The effect of aerial spray applications of Roundup® on the abundance and speciation of stream invertebrate drift has been studied previously for a number of British Columbia forest test sites.

Past field toxicity tests with herbicides have sometimes failed to measure herbicide concentrations to which the test organisms are exposed, or have used methodologies that do not assure valid results. Available data on herbicide dynamics in forest streams, following intentional direct overhead spraying, indicate that concentrations peak rapidly (within a few hours) and dissipate to extremely low levels (<10 µg/L) within 24 h post-spray. Transient peak concentrations normally do not exceed 250 µg/L. Although the use of buffer zones effectively eliminates these early spikes of herbicides within forest streams, the initial post-spray rainfall events can mobilize herbicides, resulting in brief streamwater strengths as high as (in one report) 100 µg/L.

Proposed toxicity test protocol

The tests proposed in this report for evaluating the toxic risk to regional salmonid fish stocks, posed by applications of forestry herbicides, are restricted to lethal and sublethal (acute and chronic) bioassays. Various life stages of rainbow trout and coho salmon (or an alternative Pacific salmon species) and various sensitive freshwater invertebrate species are recommended for use. Additional approaches deserving experimentation, validation and standardization before their possible inclusion as protocol methods are also presented.

The proposed tests include:

- bioassay tests to determine herbicide stability in freshwater (degree of change in acute lethal toxicity of diluted herbicide due to hydrolysis, volatilization, oxidation or other chemical alterations).
- Controlled LC50 tests to establish the time/concentration relationship for acute lethal toxic effects caused by herbicides, during and following brief exposures.
- A series of controlled tests to examine the influence of variables in the natural environment (water temperature, pH, physical and chemical characteristics of diluent or receiving waters) on herbicide toxicity.
- Controlled EC50 tests to measure the lowest herbicide strengths producing acute sublethal toxic effects (overt behavioral responses; avoidance and/or attraction responses; acute stress responses; impaired seawater tolerance) on salmonid fish.
- A 21-day complete life cycle test with *Daphnia magna* to determine the effect of herbicide exposure on chronic survival and reproduction.
- A controlled 28-day partial life cycle test with salmonid fish, designed to identify the relative sensitivity of early life stages and residual chronic toxic effects due to brief (4-day) herbicide exposures.
- Field toxicity tests including caged studies with hatchery-reared salmonid fish, mayfly and/or stonefly nymphs, adult amphipods, and *Daphnia magna*.

Pre- and post-spray field monitoring surveys of indigenous populations of salmonid fish and stream invertebrates for survival, distribution and abundance are also recommended, depending on available resources.

ACKNOWLEDGEMENTS

The sponsoring agencies (Canadian Forestry Service and B.C. Ministry of Forests) which provided the financial support for this project under the Forest Resource Development Agreement (FRDA) are gratefully acknowledged. The direction and assistance given by the Research Branch of the Ministry of Forests and, in particular, by Drs. R. Parish and T. Baker, is also acknowledged with thanks.

The following persons each provided valuable technical input to this project, and are thanked accordingly: Ms. S. von Schuckmann and Mr. R. Travers (Recreational Fisheries Branch, Ministry of Environment); Mr. J. Henigman (Protection Branch, Ministry of Forests); Mr. S. Samis (Habitat Management Division), Dr. J. Servizi (Biological Sciences Branch),Dr. L. de March (Freshwater Institute) and Dr. I. Birtwell (Fisheries Research Branch) of Fisheries and Oceans Canada; and Mr. M. Wan and Mr. R. Watts (Conservation and Protection, Environment Canada).

The agencies, companies, and individuals listed in Appendix 2 are sincerely acknowledged for their kind cooperation in providing many of the technical documents, reports, and publications reviewed in this report; as well as for their useful comments and suggestions. Without this support and cooperation, the present undertaking would not have been possible. The invaluable assistance given so willingly by our American counterparts is especially appreciated.

Finally, we wish to thank B.C. Research and, in particular, Ms. V. Esen and her staff for their significant contribution in searching and retrieving the technical literature incorporated in this report.

LIST OF ABBREVIATIONS

ACTH	adrenocorticotrophic hormone
a i	active ingredient
BAM	2,6-dichlorobenzamide
$CaCO_3$	calcium carbonate
$CaSO_4$	calcium sulfate
Cd	cadmium
cm	centimeter
Cu	copper
2,4-D	2,4-dichlorophenoxyacetic acid
2,4,5-T	2,4,5-trichlorophenoxyacetic acid
DEF	S,S,S-tributyl-phosphorotrithioate
DDT	2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane
EC50	median effective concentration
Fe	iron
h	hour
ha	hectare
H_2O	water
H_3BO_3	boric acid
KCl	potassium chloride
KH_2PO_4	potassium phosphate
kg	kilogram
km	kilometer
L	liter
LC50	median lethal concentration
LOEC	lowest observed effect concentration
m	meter
MATC	maximum acceptable toxicant concentration
meq	milliequivalent
MCPA	4-chloro-2-phenoxyacetic acid
mg	milligram
$MgSO_4$	magnesium sulfate
mL	milliliter
$NaHCO_3$	sodium bicarbonate
NaOH	sodium hydroxide
NOEC	no observed effect concentration
o/oo	parts per thousand
Pb	lead
µg	microgram
Zn	zinc
<	less than
>	greater than
≤	less than or equal to
≥	greater than or equal to

TABLE OF CONTENTS

EXECUTIVE SUMMARY iii

ACKNOWLEDGMENTS v

ABBREVIATIONS vi

1. INTRODUCTION 1

   1.1 Objectives 1
   1.2 Background 1
   1.3 Approach and Scope 2

2. LITERATURE REVIEW 3

   2.1 General 3
   2.2 Laboratory Toxicity Tests 3
      2.2.1 Acute lethal tests with salmonid fish 3
      2.2.2 Acute lethal tests with freshwater invertebrates 6
      2.2.3 Acute sublethal tests with salmonid fish 8
      2.2.4 Acute sublethal tests with freshwater invertebrates 10
      2.2.5 Chronic sublethal tests with salmonid fish 10
      2.2.6 Chronic sublethal tests with freshwater invertebrates 13
      2.2.7 Influence of environmental variables on acute toxicity 13
   2.3 Field Toxicity Tests 17
      2.3.1 Tests with salmonid fish 17
      2.3.2 Tests with freshwater invertebrates 19
   2.4 Herbicide Concentrations During Toxicity Tests 20
   2.5 Dynamics of Herbicides in Forest Streams 23

3. PROPOSED TEST PROTOCOL 26

   3.1 General Approach 26
   3.2 Acute Lethal Tests 28
      3.2.1 General 28
      3.2.2 Tests for chemical stability in water 32
      3.2.3 Comparative tests of time/concentration effects 33
      3.2.4 Tests for temperature effects 34
      3.2.5 tests for pH and receiving water effects 34
   3.3 Acute Sublethal Tests 35
      3.3.1 Overt sublethal effects 35
      3.3.2 Avoidance/preference responses 37
      3.3.3 Acute stress tests 38
      3.3.4 Salinity tolerance tests 38
   3.4 Chronic Sublethal Tests 39
      3.4.1 General 39
      3.4.2 Tests with salmonid fish 40
      3.4.3 Test with *Daphnia magna* 41
   3.5 Field Tests for Toxic Effects 41
      3.5.1 General 41
      3.5.2 Salmonid fish tests 42
      3.5.3 Invertebrate tests 43
      3.5.4 Herbicide concentrations in water 44
   3.6 Concluding Comments 44

4. REFERENCES 45

APPENDICES

1 List of data bases searched 53
2 List of contacts 55

TABLES

1 Summary of British Columbia area (ha) treated by herbicides during 1986 1
2 Summary of laboratory procedures used to evaluate the acute lethal toxicity of herbicides to salmonid fish 5
3 Summary of laboratory procedures used to evaluate the acute lethal toxicity of herbicides to freshwater invertebrates 7
4 Summary of laboratory procedures used to evaluate the acute sublethal toxicity of herbicides to salmonid fish 9
5 Summary of laboratory procedures used to evaluate the chronic sublethal toxicity of herbicides to salmonid fish 11
6 Summary of laboratory procedures used to evaluate the chronic sublethal toxicity of herbicides to freshwater invertebrates 14
7 Summary of field trials conducted to determine the influence of environmental variables on the acute lethal toxicity of herbicides to salmonid fish and freshwater invertebrates 16
8 Summary of field trials conducted to evaluate the toxic effects of herbicides towards salmonid fish and freshwater invertebrates 18
9 Summary of freshwater herbicide concentrations measured during acute lethal toxicity tests with salmonid fish and freshwater invertebrates 21
10 Summary of freshwater herbicide concentrations measured during chronic toxicity tests with salmonid fish and freshwater invertebrates 22
11 Concentrations of herbicides measured in forest streams following spray applications 25
12 Proposed test protocol for evaluating the toxic risk to regional fisheries resources posed by forest-use herbicides 29
13 Promising aquatic toxicity test procedures for forest-use herbicides requiring experimentation, validation, and standardization 31
14 Definition of fish behaviour terms 36

FIGURES

1 Apparatus for determining the avoidance and/or preference responses of salmonid fish to forest-use herbicides 37

1 INTRODUCTION

1.1 Objectives

This study had two main objectives:

1. To review the technical literature concerned with methodologies used to measure the toxic threat to salmonid fish and sensitive freshwater invertebrate species, due to the application of forestry herbicides.
2. To develop a meaningful, cost-effective bioassay protocol for evaluating the relative degree of risk to regional fisheries resources, posed by these chemicals.

1.2 Background

In British Columbia, an estimated 49 000 ha of forest land (25% of the area harvested each year) is added annually to the provincial inventory of brush sites following harvesting (Humphreys 1987). Herbicides are used in British Columbia on about 12% of the forest sites which receive some sort of silvicultural treatment (Henigman 1984). In 1986, herbicides were applied to 7935 ha to prepare harvested sites for planting; to 7848 ha to release established crop trees; and to 6150 ha to enhance crop seedling survival (Humphreys 1987). Additional applications covering less than 2000 ha were made for other purposes, such as weed seed tree control, site rehabilitation, and right-of-way maintenance.

As shown in Table 1, glyphosate (Roundup®) is presently the most commonly used herbicide in British Columbia forest sites. A total of 20 710 ha of forest was treated with glyphosate (18860 kg active ingredient) in 1986 (Humphreys 1987). Amine/ester formulations of 2,4-D (3218 kg active ingredient) were applied to 2417 ha; hexazinone (1313 kg active ingredient) was applied to 879 ha (Humphreys 1987). Quantities of other herbicides (e.g., fosamine, triclopyr) applied in British Columbia forests during 1986 were insignificant. Methods of herbicide application included aerial spraying by helicopters and fixed-wing aircraft (29% of areas treated), ground spraying using backpack or vehicle-mounted apparatus (13%), and tree injection by hack-and-squirt or other means (58%) (Table 1).

TABLE 1. Summary of B.C. forest area (ha) treated by herbicides during 1986[a]

Herbicide	Method of application			
	Aerial spray	Ground spray	Tree injection	Total (ha)
glyphosate (Roundup®)	7 031	1 750	11 929	20 710
2,4-D	16	735	1 666	2 417
hexazinone (Velpar®)	-	517	362	879
fosamine (Krenite®	-	30	-	30
TOTAL (ha)	7 047	3 032	13 957	24 036

[a] Derived from data provided by Humphreys (1987).

A portion of the forestry herbicides applied aerially or by ground spraying may enter streams by direct application to the stream surface, by accidental drift of spray, or by overland or groundwater flow during periods of intense precipitation after spraying (Lorz *et al.* 1979; Norris 1981). Depending on a number of factors (e.g., the nature and amount of chemical entering the stream; streamwater concentration; receiving water characteristics; duration of chemical exposure; chemical tolerance of exposed organisms; life stage exposed), the well-being of stream-dwelling fish and aquatic invertebrate species may be

threatened by the unintentional introduction of these chemicals. In many instances, herbicides entering streams may be sufficiently diluted and exposures brief enough that no harmful effects towards sensitive fish and other aquatic life are caused by their entry. However, under certain conditions, the same chemicals might result in sublethal toxic effects or, in the extreme case, death of the exposed organisms. Accordingly, the evaluation of new or current forestry herbicides for their apparent degree of toxicity towards freshwater aquatic life is warranted. In consideration of the high use of British Columbia forest streams by various Pacific salmon and trout species, their sensitivity to aquatic contaminants, and the need to protect this resource (Toews and Brownlee 1981), a focus of attention on freshwater-dwelling salmonid fish and their prey seems justified.

Toxic effects towards sensitive fish and freshwater invertebrates may occur rapidly (seconds, minutes, hours) upon chemical exposure, in which case they are referred to as acute effects ("acute toxicity"). Such rapid effects may be death ("acute lethal toxicity") during or within a brief period following chemical exposure; or sublethal effects ("acute sublethal toxicity"). Acute sublethal toxic effects include behavioral changes (e.g., increased or decreased activity, attraction, avoidance, loss of equilibrium, surfacing, or other actions which may increase predation on fish by birds or other predators), as well as physiological and biochemical changes (e.g., impaired seawater adaptability for smolting salmon, stress, reduced tolerance to natural environmental variables).

Other toxic effects may not be evident for weeks or months, and are referred to as chronic effects ("chronic toxicity"). These may include the delayed death of exposed organisms ("chronic lethal toxicity") or long-term sublethal toxic effects such as impaired growth, reduced disease resistance, reduced reproductive success, and diminished survival of offspring. It should be noted that chronic toxic effects are possible in instances where the duration of chemical exposure is brief (acute).

Concerns with the potential aquatic toxic impact of forestry herbicides are seasonal, inasmuch as these chemicals are applied in the spring and summer months (May through September). During this period, all freshwater life stages of salmonid fish (eggs, alevins, swimup fry, fingerlings, juveniles, adults) are present in British Columbia forest streams. Forestry herbicides are used in all of the province's forest regions (Vancouver, Prince Rupert, Prince George, Kamloops, Nelson, Cariboo), but predominantly in the Prince George, Vancouver and Kamloops regions (93% in 1986) (Humphreys 1987). Thus, site-to-site variations in streamwater quality (i.e., temperature, pH, hardness, dissolved oxygen content, soluble organic and inorganic loading, suspended solids content) occur for the various sites treated. A meaningful appraisal of the potential toxic threat towards regional fisheries resources must therefore consider the modifying influences of season, fish life stage, and receiving water quality.

1.3 Approach and Scope

The primary goal of this study is to develop a bioassay protocol for testing the toxicity of forestry herbicides, as applicable to regional fisheries resources. To this end, past studies evaluating the lethal and sublethal toxic effects of forestry herbicides towards salmonid fish and freshwater invertebrate species were reviewed and their test procedures and effects studied. The data bases searched are listed in Appendix 1, together with a list of the key and assisting words used for finding relevant material. Persons and organizations contacted with requests for current preprints, publications, and criteria documents are listed in Appendix 2.

This report summarizes toxicity test procedures used to date for evaluating the potential hazard of forestry herbicides to salmonid fish and freshwater invertebrate species. The technical literature dealing with laboratory and field bioassays (tests for acute lethal toxicity, acute sublethal toxicity, and chronic toxicity) with salmonid fish and invertebrates is reviewed under separate headings. Based on this review and in consideration of the need for a battery of aquatic toxicity tests applicable to both the regional fisheries resource and the nature of herbicide application and entry to forest streams, a test protocol for the effective evaluation of forestry herbicide toxicity is presented here.

The scope of this study is limited to review and appraisal of toxicity test procedures applicable to forestry herbicides, freshwater life stages of salmonid fish, and the latter's freshwater prey. Because there is not yet a direct association between the degree of pesticide accumulation in tissues of aquatic life and its toxic impact, the subjects of herbicide bioaccumulation, biomagnification, retention and depuration are not considered here; nor is the issue of flavor impairment due to fish exposure. The interested reader is referred to a recent and comprehensive review of these subjects by Murty (1986a). The broader issue of

herbicide chemistry and fate in the terrestrial and aquatic environment is, likewise, not a subject of this report.

2 LITERATURE REVIEW

2.1 General

During the past 20 years, considerable progress has been made in the development and standardization of aquatic toxicity test procedures. The early publications by Sprague (1969, 1970, 1971, 1973) are still worthwhile references, and form the basis of much of the toxicity procedural documents used today. Standard practices currently used by aquatic toxicologists for evaluating the toxicity of chemicals and effluents towards aquatic life are detailed in the following reports: American Public Health Association, American Water Works Association, and Water Pollution Control Federation (1985); Peltier and Weber (1985); Horning and Weber (1985); ASTM (1980); and U.S. Environmental Protection Agency (1975).

A wide variety of lethal and sublethal toxicity tests with freshwater aquatic life have been applied in efforts to define the degree and mode of toxicity caused by their exposure to chemicals or effluents. The Fisheries and Oceans Canada publication "Bibliography of Toxicity Test Methods for the Aquatic Environment" (McLean *et al.* 1980) lists 762 reports concerning diverse methodologies used for assessing the lethal and sublethal toxic effects towards fish and aquatic invertebrates, caused by their exposure to chemicals or effluents. An earlier Fisheries and Oceans Canada report "Toxicity Tests for Freshwater Organisms" (Scherer 1979) presents 20 toxicity test procedures used with Canadian freshwater organisms. Many of the approaches described in these reports, while of interest and value in assessing the potential environmental impact of certain chemicals and effluents, are not pertinent to an evaluation of the risk to regional fisheries resources due to the use of forestry herbicides.

The following publications provide current and comprehensive reviews of the toxicity of herbicides and insecticides towards freshwater fish and invertebrate species, and are of direct relevance to this study: Muirhead-Thomson (1987); Murty (1986a); Murty (1986b); and Norris, Lorz and Gregory (1983). These references provide a review of the concentrations of specific forestry pesticides shown to cause toxic effects towards freshwater aquatic life.

2.2 Laboratory Toxicity Tests

2.2.1 Acute lethal tests with salmonid fish

Acute lethal toxicity tests with laboratory-reared salmonid fish (most commonly, rainbow trout fry or young fingerlings) are conducted routinely in Canada and elsewhere. Canadian and provincial procedures for undertaking these tests have been largely standardized (Environment Canada 1980; B.C. Ministry of Environment 1982). Tests with herbicides and other chemicals determine the median lethal concentration (LC50): that is, the concentration that kills 50% of the exposed fish within a given time. A group of fish (usually 10) is placed in each of a number (usually 5 - 10) of differing chemical concentrations, and observed for percent survival at no more than 24-h intervals. The conventional period of exposure is 96 h (4 days), hence the 96-h LC50 bioassay.

Options for performing this test include the following:

static test - fish are exposed to the same solution for the duration of the test.

static replacement test - static test with periodic exposure (usually at 24-h intervals) of fish to a freshly prepared solution at the same concentration.

flow-through test - test in which solution is replaced continuously for the duration of the exposure.

The various types of laboratory procedures used for evaluating the acute lethal toxicity of herbicides to salmonid fish are summarized in Table 2. Due to its simplicity and relatively low cost, the static test is most frequently used as a measure of a herbicide's acute lethal toxicity to fish. The less frequently applied static replacement or flow-through tests are used when the stability of the chemical in solution is in question. The former procedure is accomplished best by siphoning and replacing 85 - 95% of each test solution at 24-h intervals (Lorz *et al.* 1979; McLeay and Gordon 1980a), although the occasional investigator has limited solution replacement to once only, at 48 h (Zitko *et al.* 1976). Flow-through tests are accomplished by metering fresh dilutions of a chemical into test aquaria, typically at a rate that also replaces up to 95% of each test solution every 24 h. In the few instances where LC50 values have been derived for herbicides by static versus replacement procedures and compared under otherwise identical conditions, test results have been similar (McLeay and Gordon 1979, 1980a). This is thought to reflect the stability of the herbicide in solution (in this instance, fosamine) under the test conditions used.

The duration of salmonid fish exposure to herbicides in LC50 tests has most often been according to convention (i.e., for 96 h) (Table 2). Some researchers have calculated LC50 values for exposures as short as 24 h (Folmar *et al.* 1979, Alexander et al. 1985); others have continued exposures, with or without solution replacement, for periods of up to 336 h (14 days). A few studies (Table 2) have continued to monitor fish surviving the exposure period for subsequent survival and well being, following their transfer to freshwater.

The quality of freshwater used for conducting toxicity tests with herbicides often differs from study to study, being derived from a variety of sources (such as dechlorinated municipal water supplies, lakes, rivers, groundwater) (Table 2). Some researchers have used reconstituted water -- de-ionized or distilled water to which specified amounts of reagent-grade chemicals are added (U.S. Environmental Protection Agency 1975; ASTM 1980) -- as the control and diluent water used in bioassay tests with herbicides (Table 2). The advantages of reconstituted water include its consistency (for comparative studies with other chemicals) and assured high quality; disadvantages include the high cost in preparing the necessary quantities and its "artificial" nature (e.g., absence of trace quantities of soluble organic and inorganic chemicals normally present in natural water sources). Scientists have expressed doubts that fish LC50 values derived for certain herbicides, using reconstituted water as the diluent water, are applicable to natural waters (Servizi *et al.* 1987).

Although rainbow trout are the salmonid species most commonly used for evaluating the aquatic toxicity of herbicides and other chemicals, other trout species as well as all species of salmon have been employed with success (Table 2). A number of researchers have noted that salmonid species of a similar size and life stage are generally similar in their tolerance (LC50 values) to a specific herbicide (Meehan *et al.* 1974; Servizi *et al.* 1987; Wan, Moul, and Watts 1987). However, species-specific differences in susceptibility of a particular life stage to a particular herbicide can indeed exist, as is evident when toxicity tests are conducted concurrently using the same diluent water and otherwise similar test conditions (McLeay and Gordon 1980a; Martens *et al.* 1980; Finlayson and Verrue 1985). No consistent pattern in relative herbicide susceptibility for the various species of salmonid fish frequenting British Columbia forest streams is evident at this time. It is likely that any significant differences are chemical-dependent.

Most acute lethal fish toxicity tests with herbicides have been undertaken using swimup fry or fingerling life stages (Table 2); although some researchers (Folmar *et al.* 1979; McLeay and Gordon 1980a) have tested the response of egg, alevin, smolt, and juvenile salmonid fish in efforts to determine the relative susceptibility of these life stages. Studies with a variety of aquatic contaminants have frequently indicated that, relative to the highly sensitive alevin and early free-swimming stages (swimup fry, fingerlings), fish eggs and older salmonid fish (juveniles, adults) are more resistant to toxicants. The lesser sensitivity of the eggs is attributed to the relative impermeability of the egg membrane; while the greater tolerance of the larger size/older age groups is attributed to their larger lipid pool and to a decreased gill surface area in relation to body size (Murty 1986b). Salmonid eggs have been shown to be relatively tolerant to acute exposure to certain herbicides including fosamine (Krenite®) and glyphosate (Roundup®) (Folmar *et al.* 1979; McLeay and Gordon 1980a). The few studies conducted with alevins indicate that this life stage is more sensitive to herbicides than are eggs or older free-swimming fish (Folmar *et al.* 1979; McLeay and Gordon 1980; Martens *et al.* 1980a). Limited testing with salmon smolts in freshwater indicates that this life stage is no more tolerant to herbicide exposure than are fry or young fingerlings, and perhaps more sensitive than pre-smolts or older fish (McLeay and Gordon 1980a; Finlayson and Verrue 1985). Laboratory LC50 tests with 2,4-D, fosamine, glyphosate, and triclopyr indicate that, for various species of Pacific salmon and trout, swimup fry and young fingerling salmon and trout are equally susceptible to these herbicides. Older fish (excluding the sensitive

TABLE 2. Summary of laboratory procedures used to evaluate the acute lethal toxicity of herbicides to salmonid fish

Herbicide	Test fish[a]	Life stage[b]	Test type[c]	Dilution water[d]	Duration of exposure(h)	Recovery period	Reference
2,4-D	R, C, S, CHU, DV	SF, F, S	S	L	96	no	Meehan *et al.* 1974
dinoseb	CT	SF, F	S	RC	96-240	no	Woodward 1976
2,4-D	R	F	S	DC, F	96	no	Pearce and McBride1978
2,4-D	CT, LT	F	S	RC	96	no	Woodward and Mayer 1978
glyphosate	R	E, A, SF, F	S	RC	24, 96	no	Folmar *et al.* 1979
glyphosate	R	E, A, SF	S	RC	4	yes	Folmar *et al.* 1979
Bolero 8EC®	R	–	S	RC	96	no	Sanders and Hunn 1982
DEF	R	SF	S	RC	96	yes	Palawski *et al.*1983
picloram	R	SF	S	DC, F	96	no	Mayes and Dill 1984
2,4-D, MCPA	R	SF	S	DC, F	24, 48, 72, 96	no	Alexander *et al.*1985
2,4-D, 2,4-D/oil	R	F	S	DC	48, 72, 96	no	Wan *et al* .1986
fluridone	R	–	S	–	96	no	Hamelink *et al.* 1986
glyphosate	R, C, CH	SF, F	S	DC, RC, L	96	no	Mitchell *et al.* 1986
glyphosate	R, C, S	SF, F	S	L	96	no	Servizi *et al.* 1987
glyphosate	R, C	SF, F	S	DC	24, 48, 72, 96	no	Wan, Watts and Moul 1987
Garlon®	R, CH, CHU, S, P	SF, F	S	DC	24, 48, 72, 96	no	Wan, Moul and Watts 1987
triclopyr	R, CH, CHU, S, P	SF, F	S	DC	24, 48, 72, 96	no	Wan, Moul and Watts 1987
2,4-D, dinoseb	A	F	SR	–	96	no	Zitko *et al.* 1976
2,4-D, fosamine	C	Y	SR	GW	96	no	Lorz *et al.* 1979
fosamine	C	F	S, SR	DC	96	no	McLeay and Gordon 1979
2,4-D	R, C, S, P	SF, F	SR	L	96, 168	no	Martens *et al.* 1980
fosamine	R, C	E, A, SF, F, PS, S	S, SR	DC, L, R, GW	96, 336	yes	McLeay and Gordon 1980a
endothall	CH	F	FT	L, F	336	yes	Liguori *et a* . 1983
2,4-D	R, CH	SF, S	FT	F, R	96	no	Finlayson and Verrue1985
molinate	ST, CH	SF, F	FT	F, R	96	no	Finlayson and Faggella 1985
picloram	R	–	FT	RC, DC	96, 192	no	Mayes *et al.* 1987

[a] R- rainbow trout, LT- lake trout, CT- cutthroat trout, ST- steelhead trout, A- Atlantic salmon, P- pink salmon, DV- dolly varden, C- coho salmon, S- sockeye salmon, CHU- chum salmon, CH- chinook salmon.

[b] E- egg, A- alevin, SF- swimup fry, F- fingerling, PS- pre-smolt, S- smolt, Y- yearling.

[c] S- static test, SR- static replacement test, FT- flow-through test.

[d] DC- dechlorinated, F- filtered, RC- reconstituted, GW- groundwater, L- lake, R- river.

smolt stage) have a somewhat greater tolerance (Meehan *et al.* 1974; Folmar *et al.* 1979; McLeay and Gordon 1980a; Martens *et al.* 1980; Servizi *et al.* 1987).

2.2.2 Acute lethal tests with freshwater invertebrates

Established laboratory procedures have been derived for conducting acute lethal toxicity tests with freshwater invertebrate species (U.S. Environmental Protection Agency 1975; ASTM 1980; American Public Health Assoc. *et al.* 1985). These methodology manuals each list a number of recommended species for toxicity evaluations, including the following:

- daphnids (*Daphnia magna, D. pulex*), common name "water fleas"
- amphipods (*Gammarus pseudolimnaeus, G. fasciatus, G. lacustris*), common name "scuds"
- midges (*Chironomus sp.*)
- mayflies (*Hexagenia sp., Ephemerella sp., Baetis sp.*)
- stoneflies (*Pteronarcys sp.*).

Daphnids (and, in particular, *D. magna*) are widely used in Canada and elsewhere for acute and chronic toxicity tests with chemicals and effluents. These micro-crustaceans are found in cool or warmwater lakes and streams, and often comprise a major source of food for salmonid fry and fingerlings. Daphnids are readily cultured in the laboratory, and are known to be sensitive to many chemicals and effluents. Freshwater amphipods (*Gammarus sp.*) inhabit forest streams and ponds and are used as a food source by resident salmonid fish. Immature life stages of aquatic insects (midges, mayflies, stoneflies, caddisflies) are also major food items for all salmonid fish species inhabiting freshwater streams and ponds.

As with most other chemicals, daphnids (in particular, *D. magna*) are the freshwater invertebrate species used most commonly for acute lethal toxicity tests with herbicides (Table 3). All tests reviewed were static bioassays (no replacement of test solutions). Test durations were normally 48 h, although some researchers used longer (72 or 96 h) periods of exposure. In most instances, test temperature was held at 20-22°C. Except for two studies (Scorgie and Cooke 1979; Presing 1981) which used adults, neonate (<24 h old) daphnids were used for the tests with herbicides.

Laboratory tests conducted to evaluate the acute lethal toxicity of herbicides to scuds have involved various species (*G. lacustris, G. pseudolimnaeus, G.fasciatus*). These tests have also been static bioassays, with exposure periods of 48 - 96 h and temperatures held at 12 - 21°C (Table 3). Mature amphipods were used in each.

For the two herbicide toxicity tests that used chironomids (Folmar *et al.* 1979; Hamelink *et al.* 1986), static 48-h tests were conducted at a temperature of 22°C. Midge (*Chironomus plumosus*) larvae were used in each case. No reports of acute lethal toxicity tests with larval stoneflies, mayflies, or caddisflies exposed to herbicides were found.

As in the acute lethal tests with salmonid fish, the sources of freshwater used as diluent and control water in the tests with invertebrates were diverse (Table 3). Besides various natural sources, (ditch, lake, or groundwater), a number of tests were conducted using reconstituted water. Reported water hardness values varied from 40 to 170 mg/L.

Test results using amphipods and midge larvae were reported as LC50 (median lethal concentration) values. Results for tests with daphnids were variously reported as values of LC50 or EC50 (median effective concentration; i.e., concentration causing immobilization of 50% of the daphnids).

No consistent pattern of differing species susceptibility to herbicides for these test organisms and for salmonid fish is evident from the literature reviewed. For instance, Sanders and Hunn (1982) found that rainbow trout, daphnids, and Gammarus sp. were equally sensitive (similar LC50 values) to Bolero 8EC®. Similarly, Servizi *et al.* (1987) found similar LC50 values for daphnids and sockeye salmon fry and fingerlings in laboratory tests with glyphosate (Roundup®) and triclopyr (Garlon®4). On the other hand, Mayes and Dill (1984) reported that rainbow trout fry were more sensitive to picloram than *D. magna*; whereas Alexander *et al.* (1985) found that *D. magna* were more sensitive than rainbow trout when exposed to 2,4-D. From the limited data available, the pattern of relative susceptibility of salmonid fry, fingerlings, and their prey to herbicides appears to be chemical-dependent.

TABLE 3. Summary of laboratory procedures used to evaluate the acute lethal toxicity of herbicides to freshwater invertebrates

Herbicide	Test organism	Test type	Dilution water[a]	Test temperature(°C)	Duration of exposure(h)	Reference
2,4-D, silvex	*Daphnia m.*[b]	static	GW	21	48	Sanders 1970
glyphosate	*Daphnia m.*	static	RC	22	48	Folmar *et al.* 1979
cyanatryn	*Daphnia p.*[c]	static	F, D	–	72	Scorgie and Cooke 1979
2,4-D	*Daphnia m.*	static	F, L	15, 20	24, 48, 96	Presing 1981
Bolero 8EC®	*Daphnia m.*	static	RC	21	48	Sanders and Hunn 1982
triclopyr	*Daphnia m.*	static	F, L	20	48	Gersich *et al.* 1984
picloram	*Daphnia m.*	static	RC	20	48	Mayes and Dill 1984
2,4-D, MCPA	*Daphnia m.*	static	L	20	48	Alex and er *et al.* 1985
picloram	*Daphnia m.*	static	L	20	48	Gersich *et al.* 1985
picloram	*Daphnia m.*	static	DC, L	21	48	Mayes and Oliver 1985
fluridone	*Daphnia m.*	static	–	18	48	Hamelink *et al.* 1986
dinoseb, silvex	*Daphnia m.*	static	L	20	48	Gersich and Mayes 1986
glyphosate	*Daphnia p.*	static	–	21	96	Servizi *et al.* 1987
triclopyr	*Daphnia p.*	static	–	21	96	Servizi *et al.* 1987
2,4-D, silvex	*Gammarus sp.*	static	GW	16	48	Sanders 1970
glyphosate	*Gammarus sp.*	static	RC	12	96	Folmar *et al.* 1979
Bolero 8EC®	*Gammarus sp.*	static	RC	17	96	Sanders and Hunn 1982
picloram	*Gammarus sp.*	static	RC	21	–	Mayes and Oliver 1985
fluridone	*Gammarus sp.*	static	-	18	96	Hamelink *et al.* 1986
glyphosate	*Chironomus sp.*	static	RC	22	48	Folmar *et al.* 1979
fluridone	*Chironomus sp.*	static	–	22	48	Hamelink *et al.* 1986

[a] GW - groundwater, RC- reconstituted, F- filtered, D- ditch, L- lake, DC- dechlorinated.
[b] *Daphnia magna.*
[c] *Daphnia pulex.*

2.2.3 Acute sublethal tests with salmonid fish

The literature search indicated that only a few studies have examined the acute sublethal toxic effects caused by the controlled exposure of salmonid fish to herbicides. The majority of tests reported have been based on observations of fish behavior, although tests have also been undertaken to determine if short-term herbicide exposures may cause histological, physiological, or biochemical effects (Table 4).

While conducting 96-h LC50 tests with rainbow trout swimup fry exposed to picloram, Mayes *et al.* (1987) observed surface breathing, lack of schooling behavior, and loss of equilibrium for fish held in near-lethal (0.7 LC50) herbicide concentrations. Similarly, Dodson and Mayfield (1979) reported that yearling rainbow trout, held in a range of strengths of 2,4-D to determine its 24-h LC50, became lethargic at 0.8 LC50 (Table 4). Other researchers have largely ignored or chosen not to report the presence or absence of overt changes in fish behavior during LC50 tests.

A limited number of tests with herbicides have been performed to assess whether brief (0.3 - 1 h) exposure of groups of underyearling salmonid fish to sublethal concentrations may cause significant avoidance and/or preference (fish attraction) responses. Test apparatus used was either a Y-shaped trough, with a specific herbicide strength flowing into one arm and uncontaminated freshwater into the other (Hansen *et al.* 1972), or a sharp-gradient trough with freshwater flowing into one side and a dilute herbicide concentration into the other (Scherer and Nowak 1973). The behavioral response of fish (rainbow trout or coho salmon swimup fry or fingerlings) in these systems was chemical-and concentration-dependent (Table 4). Studies with Roundup® demonstrated that significant avoidance reactions by rainbow trout did not occur until herbicide strengths approached the 96-h LC50 value (Hildebrand *et al.* 1982). On the other hand, tests with Krenite® demonstrated that this herbicide caused avoidance by coho salmon fingerlings at strengths as low as 0.03 of the 96-h LC50 value (McLeay and Gordon 1979).

Most examinations of salmonid fish for histopathological effects attributable to controlled herbicide exposure have been concerned with changes when exposures were prolonged (>4 days). McBride *et al.* (1981) found a significant enlargement of interrenal cell nuclei in sockeye salmon swimup fry exposed for 96 h to a 2,4-D concentration equivalent to 0.6 LC50; but no effect at lower strengths. This and certain other tissues (gill, liver) showed structural changes due to shorter periods of herbicide exposure (48 h), provided that the concentration exceeded the 96-h LC50 value.

A few laboratory studies have examined salmonid fish for biochemical effects due to acute exposure to sublethal concentrations of herbicides. Measurements of plasma cortisol levels in sockeye salmon smolts indicated in one instance that a 1-h exposure to 2,4-D caused a significant elevation of this hormone in the blood (i.e., stress). However, this response was only evident when the herbicide concentration exceeded its 96-h LC50 value (McBride *et al.* 1981). Unlike this finding, McLeay and Gordon (1979) found that concentrations of Krenite® as low as 0.02 of the 96-h LC50 value caused a stress response (decreased blood leucocrit value) in fingerling coho salmon within 24 h. Thebault and Decaris (1983) reported an inhibition of gill ATPase enzyme activity for adult rainbow trout held in a high sublethal concentration of 2,4-D for 96 h (Table 4).

Reports of laboratory examinations of salmonid fish for physiological effects due to brief exposures to sublethal concentrations of herbicides are also few in number; and are based on the use of salinity challenge tests. Such tests are designed to determine if exposure of salmon smolts to a range of sublethal herbicide strengths for periods as brief as 1 h can impair their subsequent survival when they are transferred to full-strength seawater. One study (Liguori *et al.* 1983) demonstrated that chinook salmon smolts held in a dilute sublethal strength of endothall (0.05 LC50) for 96 h, died within 3 days when transferred to seawater. Bouck and Johnson (1979) reported a similar finding for coho salmon smolts exposed to a dilute sublethal concentration of endothall for only 1 h; however, no mortalities were found in a duplicate study. These researchers also used salinity challenge tests to assess the impact of short-term (1 h) exposure of salmon smolts to sublethal strengths of a number of other chemicals (including 2,4-D) on their subsequent seawater tolerance. Martens *et al.* (1980) conducted salinity challenge tests with sockeye salmon smolts and pink salmon fry held in uncontaminated seawater for 96 h, following a 24-h exposure to 2,4-D and subsequent recovery overnight in freshwater. These researchers determined that herbicide strengths as high as 1.0 of the 96-h LC50 did not impair post-exposure survival of fish in seawater, nor cause observable behavioral effects.

TABLE 4. Summary of laboratory procedures used to evaluate the acute sublethal toxicity of herbicides to salmonid fish

Function affected	Herbicide	Test fish[a]	Nature of response	Test type[b]	Time[c] (h)	LOEC[d] mg/L	LOEC[d] %LC50	Reference
Behavior	picloram	R, SF	surfacing	FT	96	10.9	70	Mayes *et al.* 1987
	2,4-D	R, Y	lethargy	S	24	10.5	80	Dodson and Mayfield 1979
	2,4-D	R, SF	avoidance	FT	1	1.0	–[e]	Folmar 1976
	glyphosate	R, SF	avoidance	FT	1	10.	20	Folmar 1976
	glyphosate	R, SF	avoidance	FT	1	>10.	–	Folmar *et al.* 1979
	Krenite®	C, F	avoidance	FT	1	295.	3	McLeay and Gordon 1979
	Roundup®	R, F	avoidance	FT	0.3	50.	100	Hildebrand et al. 1982
	Roundup®	R, F	avoidance	FT	1	40.	80	Hildebrand et al. 1982
Histology	2,4-D	S, F	interrenal hypertr.	SR	96	0.3	60	McBride *et al* .1981
Physiology	endothall	CH, SM	salinity tolerance	FT	96	3.	5	Liguori *et al.* 1983
	endothall	C, SM	salinity tolerance	–	1	<5.	–	Bouck and Johnson 1979
	2,4-D	C, SM	salinity tolerance	–	1	>30.	–	Bouck and Johnson 1979
	2,4-D	P, SF	salinity tolerance	S	24	>0.5	>100	Martens *et al.* 1980
	2,4-D	S, SM	salinity tolerance	S	24	>0.5	>100	Martens *et al.* 1980
Biochem.	Krenite®	C, F	stress (leucocrit)	S	24	200.	2	McLeay and Gordon 1979
	2,4-D	S, SM	stress (cortisol)	S	1	5.	>100	McBride *et al.* 1981
	2,4-D	R, A	inhibited gill ATPase	SR	96	5.	83	Thebault and Decaris 1983

[a] R- rainbow trout, C- coho salmon, CH - chinook salmon, P- pink salmon, S- sockeye salmon, SF- swimup fry, F- fingerling, SM- smolt, Y- yearling, A- adult.
[b] S- static test, SR- static replacement test, FT- flow-through test.
[c] Duration of herbicide exposure.
[d] Lowest Observed Effect Concentration.
[e] Not determined/not given.

2.2.4 Acute sublethal tests with freshwater invertebrates

Little attention has been given to laboratory evaluations of sublethal toxic effects towards freshwater invertebrates, caused by their short-term (minutes, hours) exposure to herbicides.

During the initial day of static 72-h LC50 tests with groups of adult *Daphnia magna* exposed to a range of concentrations of cyanatryn, Scorgie and Cooke (1979) frequently counted and recorded the numbers of daphnids swimming in each solution. Depending on herbicide concentration, the percentage of daphnids swimming was reduced from control values (90 - 100% swimmers), with effects evident within 1 h for higher (lethal) concentrations and within 1 to 4 h for sublethal concentrations.

Using the same Y-shaped chamber employed for evaluating avoidance reactions of rainbow trout swimup fry, Folmar *et al.* (1979) studied the behavior of mayfly (*E. walkeri*) nymphs exposed to Roundup® for 1 h. Significant avoidance was evident at 10 mg/L but not at the lower (0.1, 1 mg/L) concentrations tested. Additional behavioral studies with mayfly nymphs exposed to Roundup® (Folmar 1978) showed similar results. Comparative behavioral testing with mayfly nymphs and rainbow trout exposed for 1 h to this and seven other herbicides indicated that, for six of the eight chemicals, the mayfly nymphs were less sensitive. Tests with 2,4-D indicated no avoidance of mayfly nymphs even when concentrations were acutely lethal.

Kersting and van der Honing (1981) examined the short-term effect of the herbicide dichlobenil on the feeding and filtering rates of *D. magna*. Groups of adult female daphnids were placed in a range of concentrations of herbicide containing a predetermined amount of *Chlorella* and, after 4 h, the amount of food remaining was recounted with a Coulter counter. Results from these studies indicated a concentration-dependent decrease in feeding and filtering rates, with a Lowest Observed Effect Concentration (LOEC) (Horning and Weber 1985) as low as 0.1 of the 48-h LC50.

Acute sublethal (respiratory) tests with adult *D. pulex* exposed for 6 - 9 h to a range of concentrations of 2,4-D and 2,4,5-T were undertaken by Sigmon (1979). Oxygen consumption rates for groups of daphnids held in each test solution were measured at 2-h intervals using a Gilson respirometer. Test results were variable, with trends to increasing respiratory rates evident due to increasing herbicide concentration as well as increasing test temperature. Significant differences due to herbicide exposure were only found for 2,4,5-T.

Using artificial streams with concrete sides and earthen bottoms, Folmar *et al.* (1979) studied the effect of three concentrations (0.02, 0.2, and 2 mg/L) of Roundup® on normal stream drift patterns of midge (*C. plumosus*) larvae. Drift nets installed at the outlet of each stream (previously colonized with midge larvae) were monitored before and after a 12-h herbicide treatment. Significant increases in stream drift were only evident for the highest concentration tested.

No other acute sublethal effect studies, in which freshwater invertebrates were exposed to herbicides for short periods, were found in the present search.

2.2.5 Chronic sublethal tests with salmonid fish

As indicated in Table 5, the laboratory test procedures used to date for evaluating the chronic sublethal toxicity of herbicides to salmonid fish are diverse. Test procedures reviewed have included all life stages (newly fertilized eggs to mature adults) and have examined the long-term toxic effects of herbicide exposures as brief as 6 h and as long as 84 days. In the instances where fish have been examined for their ability to recover in freshwater, recovery periods have varied from 10 to 100 days (Table 5).

Partial life cycle tests (American Public Health Assoc. *et al.* 1985) have frequently been performed with salmonid fish to evaluate the long-term toxic threat posed by herbicide exposure. Typically with these tests, fish eggs (freshly fertilized or eyed) or recently hatched alevins are reared for a minimum of 60 days in a range of dilute herbicide strengths (Woodward 1976; Woodward and Mayer 1978; Finlayson and Verrue 1985; Mayes and Oliver 1985; Van Leeuwen and Maas 1985). Test solutions are, in most instances, renewed daily (flow-through tests). The exposed fish groups are monitored for a number of variables including percent survival and hatchability of eggs, time to hatching, percent alevin survival, time to yolk absorption, percent deformed alevins, and survival and growth of swimup fry and young fingerlings. Final results are sometimes expressed as the Maximum Acceptable Toxicant

TABLE 5. Summary of laboratory procedures used to evaluate the chronic sublethal toxicity of herbicides to salmonid fish

Herbicide	Test fish[a]	Nature of test or response	Test type[b]	Exposure (days)	Recovery[c] (days)	LOEC[d] mg/L	LOEC[d] %LC50[e]	Reference
picloram	LT, E	partial life cycle	FT	70	0	0.035	1	Woodward 1976
dinoseb	LT, E	partial life cycle	FT	81	0	0.001	1	Woodward 1976
2,4-D	LT, A	partial life cycle	FT	60	0	0.06	10	Woodward and Mayer 1978
2,4-D	CT, A	partial life cycle	FT	60	0	0.04	8	Woodward and Mayer 1978
2,4-D	CT, A	partial life cycle	S	7	53	0.3	60	Woodward and Mayer 1978
Roundup®	RT, E, A	partial life cycle	S	0.3	–[f]	5.	>100	Folmar *et al.* 1979
Krenite®	RT, E, A, SF	partial life cycle	S	4	100	–	100	McLeay and Gordon 1980a
Krenite®	C, E, A	partial life cycle	S	4, 12	45	–	100	McLeay and Gordon 1980a
DEF	RT, SF	partial life cycle	S	4	30	0.2	80	Palawski *et al*.1983
2,4-D	RT, P, C, E, A	partial life cycle	SR	85	0	0.2	50	Martens *et al.* 1980
2,4-D	CH, E	partial life cycle	FT	86	0	0.06	20	Finlayson and Verrue 1985
picloram	RT, E	partial life cycle	FT	60	0	0.88	5	Mayes and Oliver 1985
BAM	RT, E	partial life cycle	SR	60	0	18.	8	Van Leeuwen and Mass 1985
Weedon®	RT, F	histopathology	FT	28, 84	0	0.02	2	Yokote *et al.* 1976
MCPA	ST, F	histopathology	SR	28	0	>60.	>40	Hattula *et al.* 1978
dichlobenil	RT, Y	histopathology	FT	28	0, 14	0.3	–	Wiersma-Roem *etal 19.*78
2,4-D	RT, Y	histopathology	S	8, 14	0	0.4	<10	Pearce and McBride 1978
amitrole	C, SM	salinity tolerance	FT	6	0	25.	40	Lorz *et al.* 1979
2,4-D	C, SM	salinity tolerance	FT	6	0	200	–	Lorz *et al.* 1979
endothall	CH, F	salinity tolerance	FT	14	0	<25.	<40	Liguori *et al.* 1983
Roundup®	C, SM	salinity tolerance	FT	10	0, 10	>2.8	>13	Mitchell *et al*.1987
dichlobenil	RT, Y	blood chemistry	FT	28	0, 14	>1.2	–	Wiersma-Roem *et al.* 1978
Roundup®	RT, Y	reproduction	–	0.5	30	>2.	>25	Folmar *et al.* 1979

[a] LT- lake trout, CT- cutthroat trout, RT- rainbow trout, ST- sea trout, C- coho salmon, CH- chinook salmon, P- pink salmon, E- egg, A- alevin, SF- swimup fry, F- fingerling, SM- smolt, Y- yearling

[b] FT- flow-through test, S- static test, SR- static replacement test.

[c] Duration held in uncontaminated freshwater following herbicide exposure.

[d] Lowest Observed Effect Concentration.

[e] LOEC, expressed as a percentage of the 96-h LC50 value for test fish.

[f] Not determined/not given.

Concentration (MATC); that is, the theoretical toxic threshold concentration that falls between the highest concentration showing no effect and the next highest concentration showing an effect when compared to controls (Finlayson and Verrue 1985; Mayes and Oliver 1985). Some researchers present the No Observed Effect Concentration (NOEC) and/or the Lowest Observed Effect Concentration (LOEC) as chronic toxicity test end-points (Martens *et al.* 1980; Horning and Weber 1985).

One approach for assessing the chronic impact of herbicide exposure has been to monitor short-term exposures for delayed effects during subsequent growth and development in uncontaminated freshwater (Folmar *et al.* 1979; McLeay and Gordon 1980a; Palawski *et al.* 1983) (Table 5). Woodward (1979) reported a unique approach for evaluating the impact of picloram on cutthroat trout. In an attempt to closely simulate field exposure conditions following initial herbicide application and subsequent rain events, alevins were dosed once weekly with declining concentrations of picloram throughout a 3-week period. The study continued in freshwater until day 60, to determine latent effects on fish survival and growth. Woodward (1979) noted that the LOEC using this procedure was much higher (fish more tolerant of herbicide) than was found in a previous study where exposure was continuous throughout the same stages of development.

In studies where herbicide exposures were prolonged (≥60 days), the LOEC's shown to cause chronic toxic effects towards salmonid fish were equivalent to 0.01 - 0.5 (1-50%) of the 96-h LC50 values established for the species of test fish (Table 5). For those instances where the duration of herbicide exposure was appreciably less (0.3 - 12 days) but fish were transferred to freshwater and monitored thereafter for an extended period (30 - 100 days), LOEC's were 60% of the 96-h LC50 or higher.

Histopathological investigations have not been used routinely as part of the chronic effect studies reported; and no recent (since 1978) reports of such analyses were found in the literature. Yokote *et al.* (1976) conducted laboratory tests with rainbow trout fingerlings held in reagent-grade 2,4,5-T or its commercial formulation (Weedon®), whereby a variety of tissues were removed for histological examination after continuous exposures of 28 and 84 days. Exposure to 0.02 mg/L Weedon® (concentration equivalent to 2% of the 96-h LC50) for each of these time periods caused impaired growth and pathological changes in a number of tissues, including the liver, kidney, and cardiac muscle. Unlike these findings, Hattula *et al.* (1978) found no evidence for histological change in fingerling sea trout (*Salmo trutta*) held for 28 days in concentrations of the herbicide MCPA (4-chloro-2-phenoxyacetic acid) up to 0.4 LC50. Wiersma-Roem *et al.* (1978) exposed yearling rainbow trout to a range of sublethal concentrations of dichlobenil (2,6-dichlorobenzonitrile) for 28 days. Then, or following a subsequent 14-day period of recovery in freshwater, the trout were given histopathological examinations. Pathologies were noted for gill tissue only, with no effects identified until concentrations reached 0.3 mg/L (no LC50 value was determined for the test fish). Laboratory studies with rainbow trout yearlings held in 2,4-D for 8 or 14 days found degenerative changes in liver tissue at concentrations equivalent to 0.2 - <0.1 of the 96-h LC50 (exposures of 8 and 14 days, respectively). No changes were evident in other tissues (kidney, gonad, and gut).

A few studies have reported the findings of salinity tolerance tests with Pacific salmon smolts and fingerlings, in which herbicide exposures have been sub-acute (6 - 14 days duration) (Table 5). In the laboratory investigations by Lorz *et al.* (1979) and Liguori *et al.* (1983), fish were transferred directly from the herbicide solutions to uncontaminated seawater (30 o/oo) for a minimum of 10 days, during which time they were monitored daily for survival. The seawater was either replaced daily (Lorz *et al.* 1979) or unchanged (Liguori *et al.* 1983). Liguori *et al.* (1983) compared the 10-day post-exposure survival of salmon in seawater with identically treated groups held in freshwater for the same period. According to these researchers, the seawater tolerance test proved to be a sensitive indicator of otherwise sublethal toxicity.

Mitchell *et al.* (1987) used the seawater challenge test developed by Clarke and Blackburn (1977) to assess the toxic impact of prior (10-day) exposure of freshwater-acclimated coho salmon smolts to sublethal concentrations of Roundup® on their ability to osmoregulate when transferred to seawater. Blood sodium levels from fish held in seawater for 24 h following herbicide exposure and direct seawater transfer or transfer after a 10-day recovery in freshwater, were measured and compared to those for control fish. Results indicated no effects of Roundup® concentrations equivalent to 13% or less of the 96-h LC50 value on subsequent (24-h) seawater survival or blood sodium levels; higher herbicide strengths were not examined.

Little information is available concerning the chronic effects of herbicide exposures on stress responses or other biochemical reactions within salmonid fish. Wiersma-Roem *et al.* (1978) measured blood sugar, hematocrit, and hemoglobin levels in yearling rainbow trout exposed to sublethal concentrations of dichlobenil for 28 days and examined thereafter or following a 14-day recovery in freshwater. No changes in these blood variables were evident. Test results could not be related to the 96-h LC50 value, because this test was not undertaken.

A single study (Folmar *et al.* 1979) reported examining adult salmonid fish for possible chronic toxic effects on their reproductive capability, due to herbicide exposure. In this test, mature male and female rainbow trout were exposed for 12 h to sublethal concentrations of Roundup® or glyphosate salt. Thirty days thereafter (when fish were in spawning condition), these trout were examined for gonadosomatic index (gonad weight/total body weight) and fecundity (eggs per female). No effects on these indices of reproductive potential, due to brief (12-h) exposure to Roundup® (or glyphosate salt) concentrations equivalent to 0.25 LC50 or less, were evident; higher strengths were not examined.

2.2.6 Chronic sublethal tests with freshwater invertebrates

The majority of laboratory toxicity tests undertaken to assess the chronic sublethal effects of herbicides on freshwater invertebrates are reproductive tests using daphnids (*Daphnia magna; D. pulex*) (Table 6). Seven of the nine reproductive tests reviewed employed the larger daphnid (D. magna). Test durations for these bioassays have normally been 21 - 28 days (Table 6). One study (Macek *et al.* 1976) continued the test for 64 days; however, in this instance the effect of herbicides on the survival and reproduction of three generations of daphnids was determined.

In each of the daphnid reproductive tests reviewed, test solutions were renewed by flow-through or static replacement procedures (Table 6). The frequency of static replacement varied from 1 to 3 days, with solution replacement every 2 - 3 days (e.g., Monday, Wednesday, Friday) being most common (Gersich *et al.* 1984, 1985). In all chronic effect tests with daphnid and other freshwater invertebrate species, herbicide exposures were continued throughout the test period; that is, in none of the studies were invertebrates transferred to freshwater for observations of delayed effects due to acute exposure.

In each of the chronic effect studies with daphnids, reproductive effects were judged by comparing the number of broods produced per female and/or the total number of young produced per female during the test period. Where effects were noted, these numbers were decreased due to herbicide exposure. Some investigators (Schober and Lampert 1977; Fitzmayer *et al.* 1982; Van Leeuwen and Maas 1985) reported additional chronic toxic effects during these tests (such as growth inhibition and reduced mobility). However, these were often less sensitive to herbicide exposure than were the reproductive effects.

As in the chronic effect studies with salmonid fish, final results for each herbicide have been variously reported as the MATC, the highest NOEC, and/or the LOEC. A comparison of the latter values indicates that, depending on the herbicide tested, LOEC concentrations (µg/L, mg/L) that impair the reproduction of daphnids in chronic exposure studies may differ by greater than four orders of magnitude (Table 6). Additionally, depending on the herbicide tested, the threshold chronic effect concentration may vary from 4 to 60% of the respective 48-h LC50 value (Macek *et al.* 1976; Table 6).

Hamelink *et al.* (1986) compared the chronic toxic effects of the herbicide fluridone to daphnids (*D. magna*), amphipods (*Gammarus pseudolimnaeus*) and midge larvae (*Chironomus plumosus*). All tests used flow-through conditions and the same freshwater source as diluent and control water. The test with amphipods monitored the effect of herbicide concentration on their survival and growth throughout a 60-day exposure; the chronic (30-day) test with midge larvae monitored the effect of fluridone on cumulative percent emergence. Each of these tests was less sensitive to this herbicide than was the 21-day daphnid reproductive test (Table 6).

2.2.7 Influence of environmental variables on acute toxicity

Within the freshwater environment, the toxic effect of a particular herbicide may be influenced by the specific characteristics of the receiving water. For instance, receiving water variables such as temperature, pH, dissolved oxygen content, turbidity, and the presence of natural dissolved inorganic and organic constituents (e.g., humic and fulvic acids) may modify the chemical form of the herbicide as well as the

TABLE 6. Summary of laboratory procedures used to evaluate the chronic sublethal toxicity of herbicides to freshwater invertebrates

Herbicide	Test organism	Nature of test or response	Test type[a]	Exposure (days)	LOEC[b] mg/L	LOEC[b] %LC50[c]	Reference
acrolein	*Daphnia magna*	reproduction	FT	64	0.034	60	Macek *et al.* 1976
trifluralin	*Daphnia magna*	reproduction	FT	64	0.007	4	Macek *et al.* 1976
atrazin	*Daphnia pulex*	reproduction	SR	28	1.	–[d]	Schober and Lampert 1977
2,4-D	*Daphnia magna*	reproduction	SR	21	25.	8	Presing 1981
simazine	*Daphnia pulex*	reproduction	SR	26	4.	4	Fitzmayer *et al.* 1982
triclopyr	*Daphnia magna*	reproduction	SR	21	149.	13	Gersich *et al.* 1984
picloram	*Daphnia magna*	reproduction	SR	21	18.	26	Gersich *et al.* 1985
BAM	*Daphnia magna*	reproduction	SR	21	>320.	>40	Van Leeuwen and Maas 1985
fluridone	*Daphnia magna*	reproduction	FT	21	0.4	6	Hamelink *et al.* 1986
fluridone	*Gammarus ps.*[e]	survival, growth	FT	60	1.2	29[f]	Hamelink et al. 1986
fluridone	*Chironomus p.*[g]	percent emergence	FT	30	1.2	92	Hamelink et al. 1986

[a] FT- flow-through test, SR- static replacement test.
[b] Lowest Observed Effect Concentration.
[c] LOEC, expressed as a percentage of the 48-h LC50 for test organism.
[d] Not determined/not given.
[e] *Gammarus pseudolimnaeus* (amphipods).
[f] Expressed as percentage of 96-h LC50.
[g] *Chironomus plumosus* (midge larvae).

chemical tolerance of exposed aquatic organisms (Wedemeyer *et al.* 1984; Cunningham *et al.* 1986; Madhun *et al.* 1986). The interested reader is directed to the recent review by Murty (1986b) for a detailed discussion of the influence of environmental conditions on the toxicity of pesticides and their degradation products to fish. Tests to determine the influence of environmental variables on the acute toxicity of herbicides to salmonid fish and freshwater invertebrates are described here.

A few investigators have examined the influence of temperature on the acute lethal toxicity of herbicides to salmonid fish under controlled laboratory conditions. Some tests have studied the extent to which a range of temperatures (5 - 18°C) may alter LC50 values. The magnitude of this effect, expressed as a ratio of the highest versus lowest LC50 values determined under otherwise identical conditions, is indicated in Table 7, together with specifics of the test procedures employed. These results demonstrate that, for the herbicides examined, variations of 10°C in test temperature can (depending on herbicide and fish species) cause up to a 5-fold difference in LC50 values. With the exception of 2,4-D, these tests indicated that toxicity increased (LC50 values decreased) with increasing temperatures. For instance, Roundup® was almost twice as toxic to rainbow trout at 17°C than at 7°C (Folmar *et al.* 1979). The higher toxicity of many pesticides at higher temperatures is explained on the basis of increased uptake of the toxicant due to a higher respiratory rate; yet certain pesticides (2,4-D, DDT) show an opposite response (Murty 1986b).

The effect of test temperature on the toxicity of herbicides to freshwater invertebrates has been largely ignored. Presing (1981) found that 2,4-D was slightly less toxic to daphnids (LC50 values 1.2 times higher) when acute toxicity tests were performed at 15°C rather than at the conventional 20°C test temperature for this organism. Similarly, Schultz and Kennedy (1976) noted that amitrole was only slightly less toxic to daphnids when tested in cooler water. Other reports of temperature effects on toxicity of herbicides to freshwater invertebrates were not available.

A number of studies have examined the influence of test pH on the acute lethal toxicity of herbicides to salmonid fish in freshwater (Table 7). In these studies, the pH of test solutions was adjusted to a range of values, usually representative of the pH ranges that might be encountered for natural receiving waters. The effect of test pH on LC50 values for fingerling trout was shown to be affected markedly by the herbicide. For instance, a pH range of 6.5 - 8.5 did not affect the toxicity of the two formulations (butyl ester; propylene glycol butyl ether ester) of 2,4-D examined (Woodward and Mayer 1978); whereas the same pH range caused a 33- to 43-fold variation in LC50 values derived for dinoseb (Woodward 1976). Folmar *et al.* (1979), using de-ionized water reconstituted with buffer salts, found that Roundup® was 5 times less toxic to rainbow trout fingerlings (5-fold increase in LC50 values) when tested at pH 6.5, relative to tests conducted at pH 7.5. Similarly, McLeay and Gordon (1980a) determined that Krenite® was 9 times less toxic to rainbow trout fry at pH 5.5, relative to bioassays performed at pH 7.5. Unlike these findings, the toxicity of dinoseb to cutthroat and lake trout was shown to increase markedly with decreasing pH (Woodward 1976). The effect of pH on the aquatic toxicity of herbicides and other chemicals is largely explained by the degree to which these chemicals are ionized, and by the extent to which hydrogen ion concentration affects dissociation of these compounds (Murty 1986b). Accordingly, as with temperature effects, the influence of receiving water pH on the toxicity of herbicides to fish and other freshwater life depends largely on the nature of the chemical.

The influence of water hardness on toxicity of herbicides to fish received some attention during the 1970's (Table 7). Investigators conducted LC50 bioassays with fingerling rainbow trout exposed to a variety of herbicides diluted with waters of differing hardness values (35 - 368 mg $CaCO_3$/L). In each of the tests reported, de-ionized water was reconstituted with various salt solutions, to provide the desired hardness (soft, medium, hard, very hard) without altering test pH. For the eight organic herbicides tested by Inglis and Davis (1972), diluent water hardness did not affect herbicide toxicity. Similar results were found by other researchers when testing the toxicity of picloram (Woodward 1976) and 2,4-D (Woodward and Mayer 1978); however, dinoseb was found to be twice as toxic to cutthroat and lake trout when diluted with very hard water, relative to LC50 tests performed using soft water (Woodward 1976). Murty (1986b) concluded that water hardness very rarely influences the toxicity of pesticides to fish; and suggested that many of the past observations of effects attributed to hardness were likely due to changes in pH.

TABLE 7. Summary of laboratory tests conducted to determine the influence of environmental variables on the acute toxicity of herbicides to salmonid fish and freshwater invertibrates

Environmental variable	Herbicide	Test organism[a]	Nature of test	Range of variable	Magnitude of effect[b]	Reference
Temperature (°C)	picloram	CT, LT	96 h LC50	5 - 15	1.6	Woodward 1976
	dinoseb	CT, LT	96 h LC50	5 - 15	1.4 - 3.8	Woodward 1976
	2,4-D	CT, LT	96 h LC50	5 - 15	1.4 - 1.6	Woodward and Mayer 1978
	Roundup®	RT	24 h LC50	7 - 17	1.9	Folmar *et al.* 1979
	Roundup®	RT	96 h LC50	7 - 17	1.9	Folmar *et al.* 1979
	Krenite®	RT	96 h LC50	8 - 18	4.8	McLeay and Gordon 1980a
	2,4-D	*D. magna*	48 h LC50	15 - 20	1.2	Presing 1981
pH	picloram	CT, LT	96 h LC50	6.5 - 8.5	2.1 - 2.4	Woodward 1976
	dinoseb	CT, LT	96 h LC50	6.5 - 8.5	33 - 43	Woodward 1976
	2,4-D	CT, LT	96 h LC50	6.5 - 8.5	–[c]	Woodward and Mayer 1978
	Roundup®	RT	24 h LC50	6.5 - 9.5	5.8	Folmar *et al.* 1979
	Roundup®	RT	96 h LC50	6.5 - 9.5	5.4	Folmar *et al.* 1979
	Krenite®	RT	96 h LC50	5.5 - 7.5	9.2	McLeay and Gordon 1980a
	2,4-D	RT	96 h LC50	4.5 - 8.5	>14.9	Doe *et al.* 1986
Hardness (mg/L)	2,4-D	RT	48 h LC50	53 - 368	–[c]	Inglis and Davis 1972
	picloram	CT, LT	96 h LC50	35 - 300	–[c]	Woodward 1976
	dinoseb	CT, LT	96 h LC50	35 - 300	1.8 - 2.0	Woodward 1976
	2,4-D	CT, LT	96 h LC50	40 - 300	–[c]	Woodward and Mayer 1978
Aging of test solution (days)	picloram	CT	96 h LC50	0 - 28	–[c]	Woodward 1976
	dinoseb	CT	96 h LC50	0 - 28	1.2	Woodward 1976
	Roundup®	RT	24 h LC50	0 - 7	–[c]	Folmar *et al.* 1979
	Roundup®	RT	96 h LC50	0 - 7	–[c]	Folmar *et al.* 1979
	Roundup®	ML	48 h EC50	0 - 7	–[c]	Folmar *et al.* 1979
	Krenite®	RT	LT50[d]	0 - 20	–[c]	McLeay and Gordon 1980a

[a] CT- cutthroat trout, LT- lake trout, RT- rainbow trout, ML- midge (*Chironomus plumosus*) larvae.
[b] Degree to which environmental variable influenced test result (ratio of highest/lowest LC50).
[c] No effect.
[d] Lethal time to 50% mortality.

The influence of suspended solids (turbidity) on the acute toxicity of herbicides or insecticides to aquatic life has been seldom studied. Hartman and Martin (1984) conducted 48-h LC50 tests with *Daphnia pulex* held in various concentrations of glyphosate diluted with clear water, or the water source spiked with bentonite clay to give a suspended sediment concentration of 50 mg/L. The LC50 values (3.2 mg/L with suspended sediment; 7.9 mg/L without) indicated a 2.5-fold increase in toxicity due to the presence of the sediment. These researchers attributed this effect to the adsorption of glyphosate onto the sediment particles and its subsequent ingestion by daphnids during filter feeding. Subsequent tests undertaken by Hartman and Martin (1985) with the herbicides alachlor and atrazine, using identical test procedures, showed no effect of suspended sediment on the toxicity of these herbicides to daphnids. Murty (1986b) reviewed reports of instances where suspended particles may have increased or decreased the bioavailability of pesticides to aquatic life, depending on the chemical tested and the nature of the suspended sediment.

Some laboratory studies (Table 7) have examined the influence of aging of freshwater solutions of herbicides on their subsequent acute toxicity to fish. In these tests, a range of concentrations of each herbicide is normally prepared and allowed to stand for periods of 7 - 28 days (Table 7) under test conditions (temperature, lighting, dissolved oxygen) used in the fish bioassay. The acute toxicity of these solutions is compared to that determined at the same time for fish introduced into freshly prepared (unaged) solutions. For the herbicides examined this way (Roundup®, Krenite®, picloram, and dinoseb), little if any difference in acute toxicity has been found due to aging of test solutions. Unlike these findings, a 7.5-fold decrease in the acute toxicity of the insecticide antimycin to rainbow trout was demonstrated when test solutions were aged for 16 days before fish exposure (Marking and Walker 1973). Shorter periods of aging resulted in progressively less detoxification of this pesticide.

One study attempted to compare, under controlled laboratory conditions, the degree to which a variety of natural (uncontaminated) freshwater sources used as diluent waters may modify the acute toxicity of herbicides to fish or other aquatic life. McLeay and Gordon (1980a) collected 11 natural freshwaters from various sources (lakes, rivers, groundwater, and dechlorinated drinking water) across Canada. These waters differed appreciably in pH (6.7 - 8.2), hardness, and other measured characteristics. Using each of these water sources as diluent water for separate toxicity tests, comparative 96-h LC50 bioassays with rainbow trout held in a range of concentrations of Krenite® were conducted simultaneously under otherwise identical conditions. Results showed a 12-fold variation (correlated with sample water pH, hardness, and alkalinity) in acute toxicity attributable to these water sources. Similar studies with other herbicides have not been reported.

2.3 Field Toxicity Tests

2.3.1 Tests with salmonid fish

Few field tests to assess the toxic effects of herbicides on salmonid fish within the freshwater environment have been reported. The test conditions and procedures of pertinent studies reviewed for this report are summarized in Table 8.

Pearce and McBride (1978) examined the effect of 2,4-D, applied to British Columbia lakes to control Eurasian milfoil, on caged juvenile rainbow trout. Groups of 20 - 30 fish were placed in small cages at "control" and treatment plots within the North Arm of Okanagan Lake 4 days before herbicide application. Fish survival in each cage was monitored at 0, 3, 7, 11, and 16 days post-treatment. As well, samples of liver, kidney, stomach, gill, and gonad from fish held in each cage for periods of 3 or 16 days were examined for histopathological changes. Significant mortalities of caged fish were found at both the control and test sites by day 11. The cause of these mortalities was not determined, although fish were extremely crowded in the cages and fouling with periphyton may have resulted in oxygen-deficient waters within the cages (Pearce and McBride 1978). The livers and kidneys of control fish sampled at day 16 appeared normal; pathologies described as "minor" were noted in all of the kidneys and in a few of the livers from fish sampled from the treatment plot at this time. Lorz *et al.* (1979) studied the downstream migration of yearling coho salmon smolts held in sublethal concentrations of three herbicides for periods of 4 or 15 days, and released (as branded, fin-clipped fish) to a nearby stream. Numbers of migrants were monitored at a trap 6.4 km downstream, throughout the subsequent 4-week period. No significant effects on downstream migration of fish was found for the groups of 50 - 100 individuals previously exposed to

TABLE 8. Summary of field trials conducted to evaluate the toxic effects of herbicides towards salmonid fish and freshwater invertebrates

Herbicide	Test organism[a]	Type of treatment[b]	Post-treatment observation (days)	Nature of test response monitored	Reference
2,4-D	RT	manual - L	16	survival, histopathology[c]	Pearce and McBride 1978
2,4-D/picloram	CS	FT (4,15 days)	31	downstream migration	Lorz *et al.* 1979
dinoseb	CS	FT (4,15 days)	27	downstream migration	Lorz *et al.* 1979
diquat	CS	FT (4,15 days)	27	downstream migration	Lorz *et al.* 1979
Roundup®	RT	manual - S	14	survival, behavior[c]	Hildebrand *et al.* 1982
Roundup®	CS	aerial - S	–[d]	survival, behavior[c]	Holtby and Baillie 1987
Roundup®	DM	manual - P	8	survival[c]	Hildebrand *et al.* 1980
hexazinone	SI	manual - G	210	abundance, diversity	Mayack *et al.* 1982
Roundup®	SI	aerial - S	2 - 7	drift abundance	Kreutzweiser and Kingsbury 1985

[a] RT- fingerling rainbow trout, CS- yearling coho salmon, DM- *Daphnia magna*, SI- stream invertebrates.
[b] Method of application of herbicide. L - lake, FT- flow-through bioassay, S- stream, P- pond, G- ground.
[c] Caged organisms.
[d] Not indicated.

Tordon® 101 (mixture of 2,4-D and picloram) or dinoseb. However, diquat inhibited downstream migration of fish groups exposed for 4 or 15 days at all concentrations examined (lowest strength tested, 2% of 96-h LC50).

Field toxicity tests with caged salmonid fish exposed to Roundup® (glyphosate), applied both aerially (helicopter with Simplex® spray boom) and manually (back-pack spray boom) to British Columbia forest streams, were undertaken within the University of British Columbia Research Forest (Maple Ridge, B.C.) during the summer of 1979 (Hildebrand *et al.* 1982). For the manual applications, groups of 25 hatchery-reared fingerling rainbow trout were placed in cages within each of two shallow, moderately flowing streams. Following a 28-day period for acclimation (during which time the fish were fed), fish in separate cages were exposed to dosages of Roundup® equivalent to 0 (upstream controls), 1X, 10X, and 100X the recommended application rate. Fish in each cage were observed for survival and behavior, 1 h after exposure and at twice-daily intervals throughout the subsequent 2-week period. All caged fish survived during this period, and, except for some evidence of increased swimming activity during the initial 15 min following spraying, behavioral signs of distress were not observed.

Toxicity test procedures for the field trial with caged fish exposed to aerially applied Roundup® were similar (Hildebrand *et al.* 1982). Hatchery-reared fingerling rainbow trout were transferred to cages (25 fish per cage) positioned at one upstream and two downstream sites, and allowed 30 days to acclimate to these conditions before spraying. Observations of fish survival and behavior in each cage were made within minutes of direct overhead spraying, and daily thereafter throughout a subsequent 16-day test period. As in the manual field applications, no fish mortalities occurred. Additionally, no signs of fish distress were evident immediately after spraying or throughout the subsequent 16-day period of observation (including a rainfall event on day 14).

The acute survival and behavior of caged fingerling coho salmon held in a side channel of Carnation Creek (Vancouver Island, B.C.) was studied following direct overhead spraying with Roundup® (Holtby and Baillie 1987). Exposed fish showed some transient signs of distress at 2 h after application of this herbicide. Caged fish at other sites showed no similar signs of stress. No mortalities or abnormal behavior were evident at this time for the population of coho salmon fingerlings residing in the same side channel.

Populations of juvenile coho salmon overwintering in the Carnation Creek side channel were monitored for 3 years before spraying and for 2 years after spraying. In comparison with pre-spray data, no changes in overwinter mortality rates, growth rates, or movements of salmon to or from the side channel were noted during the 2 years of observation following Roundup® application (Holtby and Baillie 1987).

2.3.2 Tests with freshwater invertebrates

Muirhead-Thomson (1987) recently reviewed the literature dealing with field trials for toxic effects of pesticides, the large majority being related to evaluations of insecticides. Few reports have been published which describe field tests conducted to determine the toxic effects of herbicide applications on resident freshwater invertebrates.

Hildebrand *et al.* (1980) reported the findings of a field toxicity test performed in the University of British Columbia Research Forest (summer of 1979), to assess whether the manual spraying of a forest pond with Roundup® would cause acute mortalities of caged *Daphnia magna* (Table 8). Precautions taken to ensure success of this trial included acclimating a population of daphnids to the stream-fed forest pond water for 3 weeks before herbicide exposure. Thereafter, three groups of 25 neonate (<24 h) daphnids were placed in soft-mesh cylinders at each of four locations (water depth <30 cm), and acclimated to transfer for a further 2 days. Following the manual spray application of Roundup® to these stations at strengths equivalent to 0 (control), 1X, 10X, and 100X normal (2.2 kg/ha) field dosage (Hildebrand *et al.* 1980), a group of daphnids at each station was removed at 2, 4, and 8 days' post-exposure and examined for survival. Survival rates at each station were consistently high (92 - 100%) and unaffected by treatment.

The impact of hexazinone on stream invertebrates was studied by Mayack *et al.* (1982). The study area consisted of five small (1 ha) adjacent watersheds within the southeastern U.S., each with an ephemeral drainage. Aquatic invertebrates in the perennial stream receiving this drainage (upstream and downstream samples) were monitored just before treatment (manual application of hexazinone pellets throughout each watershed) and for 8 months after, using a "kick" sampling technique. No major alterations in species composition or abundance of these stream invertebrates were detected.

Kreutzweiser and Kingsbury (1985) reported the findings from a field investigation of drift responses of stream invertebrates, following the aerial application of Roundup® (helicopter application with Microfoil® boom; 2.0 kg/ha) in a British Columbia research forest (Carnation Creek, Vancouver Island) during the summer of 1984. Invertebrate drift samples were collected both several days before and 2 - 7 days after treatment at hourly intervals, from two sites receiving direct overhead spray, as well as from a site with a 10-m buffer zone. None of the post-spray drift densities was significantly higher than pre-spray levels. Drift analyses suggested transient effects (increased drift) on two stream organisms, namely *Gammarus sp.* (amphipods) and *Paraleptophlebia sp.* (mayflies), attributable to the Roundup® applications.

The field toxicity tests with stream invertebrates recently undertaken in Ontario by Poirier and Surgeoner (in press), although restricted to forestry insecticides, deserves mention. This work comprised a combination of stream-side flow-through toxicity tests (using multiple trough apparatus with streamwater/insecticide dilutions), in-stream caged invertebrate survival tests, and stream invertebrate drift analyses. With the flow-through apparatus and samples of stream invertebrates (including mayflies, caddisflies, stoneflies, blackflies, and dragonflies) collected 4 h before testing, LC50 bioassays were performed using each aquatic species and each of four insecticides. These tests tried to simulate a stream dose. Duplicate groups of invertebrates were exposed to specific insecticide strengths for 1 h only, and observed frequently for survival and drift (within the test troughs) during a subsequent 47-h post-exposure period in flowing streamwater. This approach is consistent with that described by Muirhead-Thomson (1987) for evaluating the toxicity of insecticides to stream invertebrates.

Results from the stream-side field bioassay with fenitrothion were compared to the stream survival of the same invertebrate species held in cages, and to stream invertebrate drift samples, prior and subsequent to the overhead aerial application of this insecticide. Stream concentrations of fenitrothion were monitored closely following this spraying. Comparison of the caged invertebrate mortalities (100% survival of upstream controls), stream drift data, and stream fenitrothion concentrations with the stream-side bioassay results, indicated that the stream-side toxicity test accurately predicted the toxic impact of this insecticide on the stream invertebrates studied.

2.4 Herbicide Concentrations During Toxicity Tests

For the vast majority of toxicity tests reviewed in this report, researchers have based the responses noted (i.e., LC50, EC50, L0EC concentrations, etc.) on the nominal concentrations of herbicides to which the organisms were exposed. The extent to which the actual test herbicide concentrations are similar to these nominal strengths can only be assessed in those instances where suitable chemical analyses of test solutions were performed at appropriate intervals, using proven analytical techniques; and where both nominal and measured concentrations were reported.

For some of the acute lethal toxicity tests performed with salmonid fish or freshwater invertebrates exposed to herbicides under controlled conditions, herbicide concentrations in test solutions were measured (Table 9). Some of the chronic toxicity tests with these organisms have also measured chemical strengths in test solutions (Table 10). For the acute tests, no consistent sampling methodology was evident. Similarly in the chronic-exposure studies, chemical strengths in test solutions have been measured as frequently as three times per week (Gersich *et al.* 1985) and as infrequently as once every 2 weeks (Woodward and Mayer 1978), or not at all.

Overall, measured values reported for acute toxicity tests ranged from 21 to 142% of nominal strengths; those for chronic tests ranged from 40 to 142% of nominal. The higher-than-nominal measured concentrations could be attributed to errors in preparing test solutions and/or errors in analyses; lower-than-nominal strengths could reflect both of the above, as well as chemical losses due to adsorption to the test apparatus, uptake by organisms, chemical degradation/conversion, or volatility.

The chemical nature of the active ingredient in the herbicide largely determines its stability in test solutions. Some (e.g., picloram, and glyphosate) are relatively stable in dilute aqueous solutions, and show little depletion in bioassays even when these solutions are not renewed (Gersich *et al.* 1985; Servizi *et al.* 1987). Others (e.g., ester formulations of 2,4-D and triclopyr) hydrolyze rapidly to acids when mixed with water (Martens *et al.* 1980; Finlayson and Verrue 1985; Servizi *et al.* 1987). In these instances, although the total concentration of herbicide (ester plus acid) may remain relatively constant during the bioassay, substantial losses of ester forms may occur. Servizi *et al.* (1987) reported 33 - 60% hydrolysis of triclopyr ester (active ingredient of Garlon® 4) in bioassay solutions within 3 days. Wan *et al.* (1987b)

TABLE 9. Summary of freshwater herbicide concentrations measured during acute lethal toxicity tests with salmonid fish and freshwater invertebrates

Nature of test	Test type[a]	Herbicide	Test organism[b]	Exposure (days)	Herbicide measured (days)	Percentage of nominal strength[c]	Reference
LC50 - fish	S	atrazine	RT	4	1,2,3,4	70 - 100	Bathe *et al.* 1975
LC50 - fish	S	dinoseb,picloram	CT,LT	4	–[d]	–	Woodward 1976
LC50 - fish	S	2,4-D	RT	4	–	–	Pearce and McBride 1978
LC50 - fish	S	2,4-D	RT	4	–	–	Woodward and Mayer 1978
LC50 - fish	S	Roundup®,glyphosate	RT	4	–	–	Folmar *et al.* 1979
LC50 - fish	SR	atrazine	CS	6	composite	90 - 142	Lorz *et al.* 1979
LC50 - fish	SR	Krenite®	CS	6	–	–	Lorz *et al.* 1979
LC50 - fish	SR	2,4-D	CS	6	composite	72 - 128	Lorz *et al.* 1979
LC50 - fish	S,SR	Krenite®	CS	4	–	–	McLeay and Gordon 1979
LC50 - fish	S	Krenite®	RT,CS	4	–	–	McLeay and Gordon 1980a
LC50 - fish	S	2,4-D	RT,CS,PS,SS	4,7	–	–	Martens *et al.* 1980
LC50 - fish	S	DEF	RT	4	–	–	Palawski *et al.* 1983
LC50 - fish	S	picloram	RT	4	–	–	Mayes and Dill 1984
LC50 - fish	FT	2,4-D	RT,CHS	4	2	80 - 111	Finlayson and Verrue1985
LC50 - fish	S	2,4-D, 2,4-D/oil	RT	4	–	–	Wan *et al.* 1986
LC50 - fish	S	Garlon® 4	RT,CS,SS	4	3,4	85 - 114	Servizi *et al.* 1987
LC50 - fish	S	Roundup®	RS,CS,SS	4	0-4	96 - 102	Servizi *et al.* 1987
LC50 - fish	S	Roundup®	CS,CHS	4	1	70 - 87	Mitchell *et al.* 1987
LC50 - fish	S	Roundup®	RT	4	1	21 - 63	Mitchell *et al.* 1987
LC50 - invertebrate	S	Roundup®,glyphosate	DM,CP,GP	2-4	–	–	Folmar *et al.* 1979
LC50 - invertebrate	S	2,4-D	DM	1-4	–	–	Presing 1981
LC50 - invertebrate	S	triclopyr	DM	2	–	–	Gersich *et al.* 1984
LC50 - invertebrate	S	picloram	DM	2	–	–	Mayes and Dill 1984
LC50 - invertebrate	S	picloram	DM	2	0,2	105 - 112	Gersich *et al.* 1985
LC50 - invertebrate	S	silvex,dinoseb	DM	2	–	–	Gersich and Mayes 1986
LC50 - invertebrate	S	Garlon® 4,Roundup®	DP	4	–	–	Servizi *et al.* 1987

[a] S- static test, SR- static replacement test, FT- flow-through test.

[b] RT- rainbow trout, CT- cutthroat trout, LT- lake trout, CS- coho salmon, CHS- chinook salmon, PS- pink salmon, SS- sockeye salmon, DM, *Daphnia magna*, DP, *Daphnia pulex*, CP, *Chironomus plumosus*, GP, *Gammarus pseudolimnaeus*.

[c] Measured concentration, expressed as a percentage of the nominal concentration.

[d] Not measured.

TABLE 10. Summary of freshwater herbicide concentrations measured during chronic toxicity tests with salmonid fish and freshwater invertebrates

Nature of test	Test type[a]	Herbicide	Test organism[b]	Exposure (days)	Herbicide measured (days)	Percentage of nominal strength[c]	Reference
chronic - fish	FT	Weedon® (2, 4, 5-T)	RT	28,84	–[d]	–[d]	Yokote *et al.* 1976
chronic - fish	FT	picloram	CT,LT	70	–[d]	–[d]	Woodward 1976
chronic - fish	FT	dinoseb	CT,LT	81	–[d]	–[d]	Woodward 1976
chronic - fish	SR	MCPA	ST	28	–[d]	–[d]	Hattula *et al* .1978
chronic - fish	S	2,4-D	RT	8,14	–[d]	–[d]	Pearce and McBride 1978
chronic - fish	FT	2,4-D	CT,LT	60	14,28,42,56	–[e]	Woodward and Mayer 1978
chronic - fish	FT	endothall	CHS	14	1,8	74 - 142	Liguori *et al.* 1983
chronic - fish	FT	2,4-D	CHS	86	weekly	62 - 79	Finlayson and Verrue 1985
chronic - fish	SR	BAM	RT	60	–[d]	–[d]	Van Leeuwen and Maas 1985
chronic - fish	FT	picloram	RT	60	bi-weekly	90 - 100	Mayes *et al.* 1987
chronic - invertebrate	FT	acrolein	DM	64	periodically	70 - 113	Macek *et al.* 1976
chronic - invertebrate	FT	trifluralin	DM	64	periodically	40 - 51	Macek *et al.* 1976
chronic - invertebrate	SR	Atrazin®	DP	28	–[d]	–[d]	Schober and Lampert 1977
chronic - invertebrate	SR	2,4-D	DM	21	–[d]	–[d]	Presing 1981
chronic - invertebrate	SR	simazine	DM	26	–[d]	–[d]	Fitzmayer *et al.*1982
chronic - invertebrate	SR	triclopyr	DM	21	–[e]	96 - 107	Gersich *et al.* 1984
chronic - invertebrate	SR	picloram	DM	21	tri-weekly	91 - 98	Gersich *et al.* 1985
chronic - invertebrate	FT	fluridone	DM	21	weekly	–[e]	Hamelink *et al.* 1986
chronic - invertebrate	FT	fluridone	CP	30	weekly	–[e]	Hamelink *et al.* 1986
chronic - invertebrate	FT	fluridone	GP	60	weekly	–[e]	Hamelink *et al.* 1986

[a] S - static test, SR- static replacement test, FT- flow-through test.
[b] RT- rainbow trout, CT- cutthroat trout, LT- lake trout, ST- sea trout, CHS- chinook salmon, DM- D*aphnia magna*, DP- *Daphnia pulex*, CP- *Chironomus plumosus*, GP- Gammarus pseudolimnaeus.
[c] Measured concentration, expressed as a percentage of the nominal concentration.
[d] Not measured.
[e] Not calculated/not presented.

noted that concentrations of triclopyr and Garlon® 3A were reduced from nominal strengths by 33 and 41%, respectively, within 0.5 h following solution preparation. Using flow-through bioassays, Finlayson and Verrue (1985) measured up to 38% hydrolysis of ester formulations of 2,4-D within 3 h.

For both 2,4-D and triclopyr, experimental data indicate that the ester form is more toxic to fish than the acid (Martens *et al.* 1980; Finlayson and Verrue 1985; Servizi *et al.* 1987). In such instances, significant hydrolysis of ester formulations during toxicity tests could substantially decrease the toxicity of the chemical from that evident if the rate of hydrolysis were reduced.

Less-than-nominal concentrations of herbicides in bioassay solutions have sometimes been attributed, at least in part, to their adsorption onto the walls and floor of the test aquaria (Martens *et al.* 1980; Mitchell *et al.* 1986). The nature of this material (such as glass, plexiglass, polyethylene, or polypropylene), as well as the properties of the test chemical, can modify chemical adsorption and resulting bioavailability (Sharom and Solomon 1981).

Past field toxicity tests with herbicides have sometimes failed to measure chemical concentrations to which test organisms are exposed; or have, in some instances, used methodologies that do not assure valid results. No stream glyphosate residues were monitored in the caged fish or invertebrate trials conducted with Roundup® by Hildebrand *et al.* (1980; 1982). Measured concentrations of 2,4-D, picloram, dinoseb, and diquat to which yearling coho salmon were exposed for 4 or 15 days, before release and observation of downstream migration (Lorz *et al.* 1979), were based upon weekly composites of daily grab samples, stored for 2 weeks or more before extraction and analysis. Lakewater samples were collected adjacent to caged fish for analysis of 2,4-D, at intervals of 0, 0.3, 2, 3, 8, 10, and 17 days following herbicide treatment for Eurasian milfoil (Pearce and McBride 1978). Techniques for sample preservation and analysis were not indicated. Hexazinone stream residues were monitored at a single site by Mayack *et al.* (1982) in conjunction with an investigation of the impact of this herbicide on stream invertebrates. Composite 8-h water samples were taken continuously by automatic sampler for 1 month following treatment and again for 2 months in late fall. These samples were retained in the sampler until weekly composites were collected, and stored (at 4°C) for an undefined period before extraction and analysis. Stream glyphosate residues were monitored in the Carnation Creek watershed in 1984 following the aerial application of Roundup® (Feng *et al.* 1987). However, these values were not available for the preliminary appraisal of the impact of this application on stream invertebrate drift (Kreutzweiser and Kingsbury 1985).

2.5 Dynamics of Herbicides in Forest Streams

Herbicides may enter forest streams, ponds, and lakes by various routes, including: direct application; drift during aerial or ground spraying; mobilization in ephemeral stream channels following rain events subsequent to spraying; leaching and groundwater entry; and overland flow. Based on herbicide monitoring studies in Oregon State forests, the relative importance of these entry routes, as well as that of other factors modifying the degree and duration of herbicides entering forest surface waters, have been reviewed (Norris 1981; Norris and Moore 1981; Norris *et al.* 1983). Norris *et al.* (1983) concluded that aerial applications in or near aquatic zones present the greatest probability of introducing chemicals into the aquatic environment by either direct application or drift. Norris and Moore (1981) stated that, although direct application and drift are likely to result in the highest concentrations of chemicals in water, their persistence in streams is brief (due to flushing).

Exposure due to mobilization of herbicides from ephemeral stream channels, and from overland flow associated with periods of significant precipitation, would be substantially less, although the duration of exposure might be slightly longer. Leaching (if it occurs) would introduce only small amounts of chemical into the stream, although the process could be prolonged (Norris and Moore 1981).

The impact of herbicides on exposed fish and other aquatic life is determined by a number of factors including the degree of exposure. This depends on both the concentration of herbicide entering and mixing with the receiving water, and on the duration of time to which resident aquatic life is exposed. Herbicides entering forest streams, ponds, and lakes are subject to a number of rapid effects (e.g., chemical hydrolysis in receiving water; chelation to natural dissolved organic constituents such as humic and fulvic acids; adsorption to suspended inorganic and organic sediments and to bottom sediments); as well as to somewhat more gradual changes due to photochemical and microbial conversion and degradation. These modifying influences, together with hydrological variables (e.g., degree and rapidity

of mixing, dilution, and dispersion in receiving waters), will determine the nature and extent to which resident or migrant aquatic life is exposed.

The entry and dissipation of herbicides in forest streams has been monitored in numerous instances. Some of the herbicide concentrations reported after experimental applications are shown in Table 11. Available information concerning peak concentrations of herbicides in these streams, subsequent to initial rain events following spray applications, is also presented.

Norris and Montgomery (1975) monitored streamwater draining a 67-ha forested area treated by direct overhead aerial spray application of a mixture of Dicamba® (1.1 kg/ha) and 2,4-D, for Dicamba® residues. Concentrations peaked (37 µg/L; 0.037 mg/L) at 5 h and declined to undetectable levels by 38 h (Table 11). Streamwater monitoring during and following rain events which occurred within the next few days detected concentrations no higher than 9 µg/L. No Dicamba® residues were found in water samples taken up to 14 months later.

Norris and Moore (1981) reported the results of a number of studies that monitored the concentrations of 2,4-D in Oregon streams after aerial spray applications of 2,4-D low-volatile esters at a rate of 2.24 kg a.i./ha. Herbicide concentrations at a site 0.5 km downstream of a creek oversprayed, peaked at 132 µg/L (0.13 mg/L) within 2 h; stream concentrations declined to levels that did not exceed 10 µg/L by 24 h and in subsequent samplings. At a separate spray site where the creek was protected by a buffer zone of 60 - 80 m, the maximum 2,4-D concentration detected was appreciably lower (25 µg/L). This peak level occurred by 2 h; streamwater concentrations at 5 h and throughout the subsequent 7-day monitoring period did not exceed 3 µg/L (Table 11). The direct overhead spray of streams within a relatively flat and marshy area (Norris and Moore 1981) resulted in the highest 2,4-D concentration recorded following aerial applications (840 µg/L at 1 h). At this site, streamwater levels of 2,4-D remained at approximately 100 µg/L (0.1 mg/L) for the next 4 days; concentrations by 12 days were <10 µg/L.

Glyphosate residues in an Oregon forest stream receiving the direct overhead application of this herbicide at a rate of 3.3 kg/ha were reported by Newton *et al.* (1984). Streamwater, monitored on the downstream edge of the site, contained glyphosate residues of 100 - 250 µg/L (0.1 - 0.3 mg/L) during the initial 5 h following spraying; glyphosate values declined to approximately 10 µg/L at 3 days and were below the limit of detection at days 6, 7, 14, 28, and 55. Significant rainfall events were recorded at days 2, 45, and 51 after treatment.

Environment Canada monitored a number of British Columbia forest streams for persistence of glyphosate following spray applications of Roundup® (Wan 1986). Streamwater collected at the downstream edge of a site in the University of British Columbia Research Forest, which had received direct overhead aerial application of this herbicide (3 kg a.i./ha), contained 20 and 30 µg glyphosate/L at 1 and 3 h, respectively. Concentrations declined to 5 µg/L by 24 h and were below the limit of detection (<5 µg/L) at days 2 and 3, although a level of 100 µg/L was evident following the first significant rainfall event (day 8). At other sites where creeks were protected by buffer zones of 50 - 100 m, aerial applications at rates of 1.5 kg/ha (Cogburn Creek) or 3 kg/ha (Chilliwack) resulted in no measurable concentrations of glyphosate in adjacent downstream waters within 3 days post-treatment (Wan 1986). At one of these sites (Chilliwack), a peak concentration of 25 µg/L was detected following several rainstorms post-spray (day 21). No glyphosate concentrations were found in streamwater samples from the Cogburn Creek site taken up to 7 days post-spray (including a major rainstorm event). Wan (1986) also monitored streamwater draining a watershed (Pender Harbour, B.C.) treated manually by backpack spraying of Roundup® (0.5 - 1.5 kg/ha). A 10-m buffer zone was adhered to along this stream. No measurable concentrations of glyphosate were found in any streamwater samples taken up to 190 days post-spray. Additionally, analyses of streamwater samples from each of the above four studies for amino-methyl-phosphonic acid (the initial degradation product from the metabolism of glyphosate) were unable to detect its presence in any samples.

Norris *et al.* (1987) recently reported the persistence of triclopyr in streamwater following its aerial application (as the isopropylamine salt) to an Oregon pasture, at a rate of 3.4 kg a.i./ha. The intermittent stream was oversprayed in May. Triclopyr concentrations in streamwater peaked at 95 µg/L within 1 h post-spray and declined to 10 µg/L by 5 h, with values of only 2 - 3 µg/L at days 1 and 2 post-spray (Table 11). The first rainfall event post-spray resulted in a post-rain streamwater concentration of 35 µg/L; the first major rainfall in September caused a transient elevation of triclopyr to 15 µg/L (Norris et al. 1987). Subsequent sampling for up to 13 months post-spray indicated no detectable streamwater concentrations of triclopyr.

TABLE 11. Concentrations of herbicides measured in forest streams following spray applications

Herbicide	Application rate (kg/ha)[a]	Buffer zone (m)	Measured stream concentration (µg/L) hours 1	2	3	5	days 1	2	3	4	Peak post-rain concentration[b] (µg/L)	Reference
Dicamba®	1.1	–[c]	ND[d]	1	3	37	6	ND	2	4	9	Norris and Montgomery 1975
2,4-D ester	2.2	–[c]	–[e]	132	–[e]	85	3	9	8	–[e]	–[e]	Norris and Moore 1981
2,4-D ester	2.2	60-80	–[e]	25	–[e]	1	–[e]	–[e]	3	–[e]	–[e]	Norris and Moore 1981
2,4-D ester	2.2	–[c]	840	-e	128	–[e]	91	76	–[e]	115	ND	Norris and Moore 1981
glyphosate	3.3	–[c]	250	220	130	100	–[e]	-e	10	–[e]	–[e]	Newton *et al.* 1984
glyphosate	3.	–[c]	20	–[e]	23	–[e]	5	ND	ND	–[e]	100	Wan 1986
glyphosate	3.	60-100	–[e]	–[e]	–[e]	ND	ND	–[e]	ND	–[e]	25	Wan 1986
glyphosate	0.5-1.5[f]	10	ND	–[e]	–[e]	–[e]	ND	–[e]	–[e]	ND	ND	Wan 1986
triclopyr	3.4	–[c]	95	50	20	10	3	2	–[e]	ND	35	Norris *et al.* 1987

[a] Rate of application of herbicide (kg active ingredient/ha).
[b] Highest concentration detected after initial rainfall event(s) subsequent to spraying.
[c] No buffer zone used (stream oversprayed).
[d] Not detectable by analytical procedure used.
[e] Not measured.
[f] Applied manually by backpack spray. All other applications aerially by helicopter.

Based on the foregoing studies, it is apparent that the concentrations of herbicides in forest streams receiving direct applications from overhead spraying or drift will be transiently (hours) elevated -- although in most instances these will be reduced to extremely low levels (<10 µg/L) by 24 h post-spray. Available information indicates that these "spike" concentrations normally do not exceed 250 µg/L (Table 11). However, higher concentrations are likely if the application rate exceeds 3 kg/ha, or if application sites are marshy areas with slow-moving water. The use of buffer zones effectively eliminates these initial (within 24 h post-spray) spikes of herbicides entering receiving waters.

Rain events can mobilize sprayed herbicides, resulting in their entry into forest streams. Existing data suggest that, in those instances where streamwater entry of herbicides has occurred following rainstorms, concentrations have again appeared as spikes or pulses, and return to very low or undetectable levels within perhaps 24 h. The likelihood of rain flushing herbicides into forest streams depends upon many environmental factors, including the nature of the herbicide and its affinity for soils and litter, its rate of degradation at the site sprayed, forest slope, and overspraying of ephemeral streams. One of the stream-monitoring studies by Wan (1986) indicated a spike of glyphosate entering a forest stream at a concentration of 100 µg/L, 8 days post-spray. No higher post-rain stream concentrations of this or other herbicides have been reported for any of the sites monitored (Table 11).

Data concerned with measured concentrations of forest herbicides in surface waters, together with aquatic toxicity data for these and other herbicides, indicate that the likelihood of aerial and manual spray applications causing acute mortalities of exposed salmonid fish and fish-food organisms is very low. This is particularly true for herbicides with laboratory LC50 values >1 mg/L, where:

1) a 10-m "pesticide-free zone" adjacent to water bodies is adhered to as specified in the province's Pesticide Use Permits (Adams 1987);

2) application rates do not exceed 3 kg a.i./ha; and

3) accidental spills do not occur. A greater risk of acute lethal toxic effects towards fish exists in shallow-water ponds and marshy areas where herbicides with LC50 values <1 mg/L are applied (Woodward 1976; Woodward and Mayer 1978; Finlayson and Verrue 1985).

The sublethal toxicity data reviewed, whereby herbicide exposures were acute (hours, few days), show threshold effect concentrations >1 mg/L in most instances (Table 4). Thus, little if any acute sublethal or lethal toxic threat towards salmonid fish, due to prescribed applications of forest herbicides (Adams 1987), has been demonstrated. However, since existing and prospective herbicides have not been evaluated thoroughly for possible acute sublethal effects, it is premature to assume that all herbicides will be harmless to salmonid fish and other freshwater life when applied in a prescribed manner. Few meaningful attempts have been made to define the threshold sublethal concentrations of these chemicals harmful to salmonid fish when exposures are brief.

The chronic sublethal toxicity data reviewed indicate numerous findings of threshold effect herbicide concentrations <1 mg/L, with several studies reporting effects at strengths <0.1 mg/L (<100 µg/L) (Tables 5 and 6). Although the herbicide-monitoring surveys indicate that stream concentrations ≥0.1 mg/L can occur (Table 11), it is evident that these levels are seldom if ever sustained for extended periods. Furthermore, since most of the chronic-effect data reported are derived from studies where fish and freshwater invertebrates were exposed to elevated herbicide strengths for weeks or months, the relevance of these findings to native fish and invertebrate species receiving typical environmental exposures to these chemicals is unclear. The potential long-term impact of forest herbicides on freshwater life would be better apprised by prolonged observations of field-dosed fish and fish-food organisms, together with controlled observations of chronic effects due to acute sublethal exposures.

3 PROPOSED TEST PROTOCOL

3.1 General Approach

Before the 1970's, test procedures for determining the toxicity of new and current-use chemicals and effluents towards aquatic life were unsophisticated. They consisted primarily of acute survival tests using readily available fish species (guppies, goldfish, tropical "pet" fish, and rainbow trout). Research scientists in British Columbia and across Canada developed test procedures in the 1970's that were more sensitive

and applicable to salmonid fish and other aquatic life frequenting local waters. The 1980's have seen the birth of the concept of "ecotoxicology" (i.e., the science of the integrated assessment of toxic aggression on all biological communities of the environment), combined with greater efforts towards developing and standardizing diverse bioassay test procedures using a variety of sensitive aquatic organisms (Agriculture Canada *et al.* 1984; Blaise 1984). According to Murty (1986b):

> Present-day aquatic toxicity testing is concerned more with the protection of diverse aquatic organisms, rather than with the enumeration of the lethal and sublethal effects of one toxicant on a single species.

The registration of herbicides and insecticides for use in Canada is administered by Agriculture Canada under the authority of the Pest Control Products Act. The pesticide review and regulatory process involves consultation with, and provision of, expert opinion from federal departments, such as Environment Canada, Fisheries and Oceans Canada and Health and Welfare Canada. It also includes diverse research, monitoring, and surveillance programs and activities (Agriculture Canada *et al.* 1984). Guidelines for determining the environmental chemistry and fate of pesticides were prepared recently by Agriculture Canada *et al.* (1987). As yet, no protocols or guidelines have been developed in Canada for the generation of aquatic species risk data associated with the evaluation of in-use or prospective pesticides (M.P. Stewart, pers. comm., 1987, Agric. Can.). Rather, aquatic toxicity data derived by the chemical manufacturing company as part of their product development programs or in response to specific requests from Ottawa, are reviewed in conjunction with toxicity data assimilated and derived by various federal departments and their regional laboratories.

The approach taken here in developing a bioassay protocol for evaluating the toxic risk to fisheries resources posed by forest-use herbicides, is to focus on those tests and test organisms that are relevant to the problem at hand. The battery of tests proposed is restricted to those directly applicable to the protection of the regional fisheries resources placed at some degree of risk by the planned application of herbicides within British Columbia forests (i.e., freshwater life stages of Pacific salmon and trout and their invertebrate prey).

The following tests have been considered and rejected from this protocol:

- Microtox® luminescent bacterial test
- algal bioassays
- microcosm assays

The Microtox® test determines the median effective concentration of chemical or effluent, which inhibits light production by the luminescent marine bacterium, *Photobacterium phosphoreum*. This test has a number of attractive features, including its simplicity, rapidity, small sample volume requirements, and reproducibility (Chang *et al.* 1981; Qureshi *et al.* 1982). Test procedures for performing the Microtox® assay are reasonably well standardized (Ribo and Kaiser 1987) and regional capabilities exist for undertaking this test. The Microtox® test has a proven value for a number of applications, including the screening of large numbers of samples of chemicals and effluents for relative toxicity, and identifying in-plant sources of effluent toxicity. Nevertheless, it is excluded from this protocol because of the nature of the test organism (marine bacterium), a test sensitivity no greater than that for LC50 tests with daphnia or salmonid fish as test organisms (DeZwart and Sloof 1983), and a limited field versatility and apparent inability to readily distinguish interactions between toxicants and receiving (diluent) water (Dutka and Kwan 1981; Ribo and Kaiser 1987).

A number of diverse bioassay procedures are available for conducting aquatic toxicity tests using freshwater algal species (OECD 1984; Sergy 1987). The algal (*Selenastrum capricornutum*) growth test method prescribed by the U.S. Environmental Protection Agency (Horning and Weber 1985) has gained acceptance in Canada and the U.S. as a suitable laboratory test procedure for measuring the toxicity of chemicals and effluents towards freshwater phytoplankton. The assured protection of primary producers within the freshwater environment is of interest insofar as they play a role in the aquatic food chain. Nonetheless, their direct relevance to the well-being of salmonid fish residing in forest streams, ponds, and lakes is questionable and of lesser importance than the assured protection of the freshwater invertebrate species upon which salmon and trout feed. Many of the aquatic invertebrate species that comprise a large portion of the diet of salmonid fish are themselves opportunistic feeders (e.g., daphnids, gammarid amphipods, chironomid larvae) or detritivores (e.g., mayfly or stonefly nymphs) (Lawrence

1981). Consequently, they do not depend entirely, if at all, on phytoplankton for their food source. Terrestrial invertebrates also form a portion of the diet of these fish. Any selective impact of a forest herbicide on freshwater algae would likely be short-lived because of recolonization; and would not be expected to place the fisheries resource at risk due to its depletion. For these reasons, and with an intent to restrict the recommended test procedures to those of direct relevance to the fisheries resource, algal bioassays are not included in the proposed protocol.

"Microcosm" or "model ecosystem" tests have been developed and applied by a number of researchers, in attempts to make progress in extrapolating laboratory testing to the field. An array of designs and procedures for undertaking model ecosystem tests have been used to examine the fate and effect of environmental contaminants on ecosystem components (flora, fauna, soil, water, sediment) (Taub 1976; Isensee 1980; Giesy 1985; Neuhold 1986). Canadian researchers have largely ignored the microcosm assay, and few attempts have been made to adapt this test to organisms and conditions representative of the Canadian environment. The use of artificial community streams and the model ecosystem, as applied to pesticides, has been reviewed recently by Muirhead-Thomson (1987). Most often, microcosm tests with pesticides have attempted to simulate pond environments (Yockim *et al.* 1980; Francis *et al.* 1985; Dewey 1986). A review of the literature by Giesy (1985) revealed no evidence that microcosm (multi-species) tests were more or less sensitive than a battery of single-species tests. The accuracy of microcosm tests in predicting ecosystem effects is presently in question, on the grounds that not all the components of the ecosystem are necessarily present, thus causing a different response of the chemical in the microcosm than what might occur in the environment (Neuhold 1986).

A growing body of evidence shows that a battery of single-species toxicity tests (lethal and sublethal; acute and chronic), if thoughtfully selected, can adequately predict the impact of pesticides and other environmental contaminants on sensitive aquatic life in nature (Hansen and Garton 1982; Adams *et al.* 1983; Chapman 1983; Mount *et al.* 1984; Poirier and Surgeoner 1988). This approach is incorporated in this protocol, considered to be suitable for evaluating the hazard of forestry herbicides to regional freshwater fisheries resources. As appropriate, certain test organisms and procedures recently recommended by Environment Canada scientists familiar with aquatic toxicity tests (Sergy 1987) have been included. Existing regional toxicity testing capabilities have also been considered during this protocol design. As well, tests selected have been chosen in consideration of their sensitivity, reproducibility, biological relevance, and cost-effectiveness; and for their ability to provide a clearer definition of potential herbicide impacts due to various time-concentration interactions.

Specific toxicity test types and procedures recommended for inclusion in the proposed bioassay protocol are listed in Table 12. Promising new approaches and procedures requiring experimentation, validation, and standardization before their possible inclusion as part of a revised protocol are outlined in Table 13.

3.2 Acute Lethal Tests

3.2.1 General

Acute lethal tests are the cornerstone of toxicity risk assessments with pesticides and other aquatic contaminants. The LC50 test provides a rapid, cost-efficient method to measure: the relative toxicity of different herbicides and their formulations; the relative sensitivity of differing aquatic species and life stages to particular herbicides; and the modifying influence of receiving water characteristics and environmental variables on chemical toxicity. Although this test cannot accurately predict threshold sublethal concentrations of herbicides harmful to salmonid fish and other sensitive aquatic species, it provides a means of expressing threshold sublethal effect concentrations (e.g., EC50, LOEC, NOEC, MATC) derived for a particular chemical, as a fraction of the median lethal concentration (e.g., 0.005 LC50, 0.1 LC50, etc.). If the lethal and sublethal tests are conducted in a manner applicable to real-world situations, these data, together with that from field toxicity tests and the monitoring of receiving waters for herbicide concentrations following test spraying operations, can provide an environmentally relevant means for assessing the potential toxic impact to aquatic life caused by prospective herbicides or particular field applications.

TABLE 12. Proposed test protocol for evaluating the toxic risk to regional fisheries resources posed by forest-use herbicides

Class of test	Test organism	Life stage[a]	Test type[b]	Response or effect measured	Length of exposure	Length of recovery	End-point
Acute lethal	rainbow trout	SF or F	FT,S	stability of test solutions	96 h	0 h	96-h LC50
	coho salmon	SF or F	FT,S	stability of test solutions	96 h	0 h	96-h LC50
Acute lethal	rainbow trout	SF or F	S	solutions aged 0 days (fresh)	96 h	0 h	96-h LC50
	rainbow trout	SF or F	S	solutions aged 7 days	96 h	0 h	96-h LC50
Acute lethal	rainbow trout	SF or F	S	time/concentration effects	1 h	95 h	96-h LC50
	rainbow trout	SF or F	S	time/concentration effects	2 h	94 h	96-h LC50
	rainbow trout	SF or F	S	time/concentration effects	6 h	90 h	96-h LC50
	rainbow trout	SF or F	S	time/concentration effects	24 h	72 h	96-h LC50
	rainbow trout	SF or F	S	time/concentration effects	96 h	0 h	96-h LC50
Acute lethal	coho salmon	SF or F	S	time/concentration effects	1 h	95 h	96-h LC50
	coho salmon	SF or F	S	time/concentration effects	2 h	94 h	96-h LC50
	coho salmon	SF or F	S	time/concentration effects	6 h	90 h	96-h LC50
	coho salmon	SF or F	S	time/concentration effects	24 h	72 h	96-h LC50
	coho salmon	SF or F	S	time/concentration effects	96 h	0 h	96-h LC50
Acute lethal	*Daphnia magna*	neonate	S	time/concentration effects	1 h	47 h	48-h LC50
	Daphnia magna	neonate	S	time/concentration effects	2 h	46 h	48-h LC50
	Daphnia magna	neonate	S	time/concentration effects	6 h	42 h	48-h LC50
	Daphnia magna	neonate	S	time/concentration effects	24 h	24 h	48-h LC50
	Daphnia magna	neonate	S	time/concentration effects	48 h	0 h	48-h LC50
Acute lethal	rainbow trout	SF or F	S	temperature effect (10C test)	96 h	0 h	96-h LC50
	rainbow trout	SF or F	S	temperature effect (15C test)	96 h	0 h	96-h LC50
	rainbow trout	SF or F	S	temperature effect (20C test)	96 h	0 h	96-h LC50
Acute lethal	*Daphnia magna*	neonate	S	temperature effect (10C test)	48 h	0 h	48-h LC50
	Daphnia magna	neonate	S	temperature effect (15C test)	48 h	0 h	48-h LC50
	Daphnia magna	neonate	S	temperature effect (20C test)	48 h	0 h	48-h LC50
Acute lethal	rainbow trout	SF	S	pH effect (pH 6.4-6.8[c])	96 h	0 h	96-h LC50
	rainbow trout	SF	S	pH effect (pH 7.2-7.6[c])	96 h	0 h	96-h LC50
	rainbow trout	SF	S	pH effect (pH 7.4-7.8[c])	96 h	0 h	96-h LC50
	rainbow trout	SF	S	pH effect (pH 7.6-8.0[c])	96 h	0 h	96-h LC50
Acute lethal	rainbow trout	SF	S	receiving-water effect (8 - 10 natural waters as diluent water for LC50)	96 h	0 h	96-h LC50
	rainbow trout	SF or F	S	time/concentration effects	1 h	95 h	96-h LOEC[d]
Acute sublethal	rainbow trout	SF or F	S	time/concentration effects	2 h	94 h	96-h LOEC
	rainbow trout	SF or F	S	time/concentration effects	6 h	90 h	96-h LOEC
	rainbow trout	SF or F	S	time/concentration effects	24 h	72 h	96-h LOEC
	rainbow trout	SF or F	S	time/concentration effects	96 h	0 h	96-h LOEC
Acute sublethal	coho salmon	SF or F	S	time/concentration effects	1 h	95 h	96-h LOEC
	coho salmon	SF or F	S	time/concentration effects	2 h	94 h	96-h LOEC
	coho salmon	SF or F	S	time/concentration effects	6 h	90 h	96-h LOEC
	coho salmon	SF or F	S	time/concentration effects	24 h	72 h	96-h LOEC
	coho salmon	SF or F	S	time/concentration effects	96 h	0 h	96-h LOEC

TABLE 12. (continued)

Class of test	Test organism	Life stage[a]	Test type[b]	Response or effect measured	Length of exposure	Length of recovery	End-point
Acute sublethal	rainbow trout	SF or F	FT	avoidance/preference behavior	1 h	0 h	1-h EC50[e]
	coho salmon	SF or F	FT	avoidance/preference behavior	1 h	0 h	1-h EC50
Acute sublethal	rainbow trout	F	S	stress	1 h	23 h	24-h EC50
	rainbow trout	F	S	stress	24 h	0 h	24-h EC50
	coho salmon	F	S	stress	1 h	23 h	24-h EC50
	coho salmon	F	S	stress	24 h	0 h	24-h EC50
Acute sublethal	coho salmon	S	S	salinity tolerance	1 h	0 h	1-h EC50
	coho salmon	S	S	salinity tolerance	1 h	95 h	96-h EC50
	coho salmon	S	S	salinity tolerance	24 h	0 h	24-h EC50
	coho salmon	S	S	salinity tolerance	24 h	72 h	96-h EC50
Chronic sublethal	rainbow trout	GE	S/FT	partial life cycle	4 days	SF + 28 days[f]	LOEC[d]
	rainbow trout	EE	S/FT	partial life cycle	4 days	SF + 28 days	LOEC
	rainbow trout	A	S/FT	partial life cycle	4 days	SF + 28 days	LOEC
	rainbow trout	SF	S/FT	partial life cycle	4 days	SF + 28 days	LOEC
Chronic sublethal	coho salmon	GE	S/FT	partial life cycle	4 days	SF + 28 days	LOEC
	coho salmon	EE	S/FT	partial life cycle	4 days	SF + 28 days	LOEC
	coho salmon	A	S/FT	partial life cycle	4 days	SF + 28 days	LOEC
	coho salmon	SF	S/FT	partial life cycle	4 days	SF + 28 days	LOEC
Chronic sublethal	*Daphnia magna*	neonate	SR	life cycle	21 days	0 days	LOEC
Field bioassay	rainbow trout	F	*in situ*	caged survival/behavior	–[g]	–	TCE[h]
	coho salmon	F	*in situ*	caged survival/behavior	–	–	TCE
	mayfly,stonefly	nymph	*in situ*	caged survival/behavior	–	–	TCE
	Gammarus sp.	adult	*in situ*	caged survival/behavior	–	–	TCE
	Daphnia magna	neonate	*in situ*	caged survival	–	–	TCE
Field sampling	salmonid sp.	varied[i]	*in situ*	survival/behavior/abundance	–	–	TCE
	invertebrate sp.	varied	*in situ*	drift abundance/speciation	–	–	TCE

[a] SF- swimup fry, F- fingerling, S- smolt, GE - green (freshly fertilized) egg, EE- eyed egg, A- alevin.
[b] FT- flow-through test, S- static test, S/FT - static exposure with flow-through post-exposure, SR- static replacement test.
[c] Peltier and Weber 1985.
[d] Lowest Observed Effect Concentration.
[e] Median Effective Concentration.
[f] Test terminated when swimup fry reared for 28 days in flowing freshwater.
[g] Dependent on field conditions and procedures employed.
[h] Time/Concentration Effect.
[i] Dependent on resident organisms.

TABLE 13. Promising aquatic toxicity test procedures for forest-use herbicides requiring experimentation, validation and standardization

Class of test	Test organism	Life stage	Test type[a]	Response or effect measured	Length of exposure	Length of recovery	End-point
	Gammarus sp	adult	S	time/concentration effects	1 h	95 h	96-h LC50[b]
Acute	*Gammarus sp.*	adult	S	time/concentration effects	2 h	94 h	96-h LC50
lethal	*Gammarus sp.*	adult	S	time/concentration effects	6 h	90 h	96-h LC50
	Gammarus sp.	adult	S	time/concentration effects	24 h	72 h	96-h LC50
	Gammarus sp.	adult	S	time/concentration effects	96 h	0 h	96-h LC50
	Hexagenia sp.	nymph	FT	time/concentration effects	1 h	47 h	48-h LC50
Acute	*Hexagenia sp.*	nymph	FT	time/concentration effects	2 h	46 h	48-h LC50
lethal	*Hexagenia sp.*	nymph	FT	time/concentration effects	6 h	42 h	48-h LC50
	Hexagenia sp.	nymph	FT	time/concentration effects	24 h	24 h	48-h LC50
Acute	*Daphnia magna*	neonate	S	temperature effect (10C test)	48 h	0 h	48-h LC50
lethal	*Daphnia magna*	neonate	S	temperature effect (15C test)	48 h	0 h	48-h LC50
	Daphnia magna	neonate	S	temperature effect (20C test)	48 h	0 h	48-h LC50
	Gammarus sp.	adult	S	time/concentration effects	1 h	95 h	96-h EC50[c]
Acute	*Gammarus sp.*	adult	S	time/concentration effects	2 h	94 h	96-h EC50
sublethal	*Gammarus sp.*	adult	S	time/concentration effects	6 h	90 h	96-h EC50
	Gammarus sp.	adult	S	time/concentration effects	24 h	72 h	96-h EC50
	Gammarus sp.	adult	S	time/concentration effects	96 h	0 h	96-h EC50
	Hexagenia sp.	nymph	FT	time/concentration effects	1 h	47 h	48-h EC50
Acute	*Hexagenia sp.*	nymph	FT	time/concentration effects	2 h	46 h	48-h EC50
sublethal	*Hexagenia sp.*	nymph	FT	time/concentration effects	6 h	42 h	48-h EC50
	Hexagenia sp.	nymph	FT	time/concentration effects	24 h	24 h	48-h EC50
	rainbow trout	fingerling	S	stress/challenge	1 h	0 h	1-h EC50
Acute	rainbow trout	fingerling	S	stress/challenge	24 h	0 h	24-h EC50
sublethal	rainbow trout	fingerling	S	stress/challenge	1 h	95 h	96-h EC50
	rainbow trout	fingerling	S	stress/challenge	24 h	72 h	96-h EC50
	coho salmon	fingerling	S	stress/challenge	1 h	0 h	1-h EC50
Acute	coho salmon	fingerling	S	stress/challenge	24 h	0 h	24-h EC50
sublethal	coho salmon	fingerling	S	stress/challenge	1 h	95 h	96-h EC50
	coho salmon	fingerling	S	stress/challenge	24 h	72 h	96-h EC50
Field	rainbow trout	fingerling	*in situ*	post-exposure stamina	–[d]	–	TCE[e]
bioassay	coho salmon	fingerling	*in situ*	post-exposure stamina	–	–	TCE
	rainbow trout	fingerling	S	field survival/behavior	1 h	95 h	96-h LC50
Field	rainbow trout	fingerling	S	field survival/behavior	24 h	72 h	96-h LC50
bioassay	coho salmon	fingerling	S	field survival/behavior	1 h	95 h	96-h LC50
	coho salmon	fingerling	S	field survival/behavior	24 h	72 h	96-h LC50
	Gammarus sp.	adult	S	field survival/behavior	1 h	95 h	96-h LC50
Field	*Gammarus sp.*	adult	S	field survival/behavior	24 h	72 h	96-h LC50
bioassay	*Hexagenia sp.*	nymph	FT	field survival/behavior	1 h	47 h	48-h LC50
	Hexagenia sp.	nymph	FT	field survival/behavior	24 h	24 h	48-h LC50
Field	salmonid sp.	varied[f]	*in situ*	histology/stamina	–	–	TCE
sampling	invertebrate sp.	varied	*in situ*	stamina	–	–	TCE

[a] S- static test, FT- flow-through test.
[b] Median Lethal Concentration.
[c] Median Effective Concentration.
[d] Dependent on field conditions and procedures employed.
[e] Time/Concentration Effect.
[f] Dependent on resident organisms.

In terms of utility for assessing the hazard of contaminants to aquatic environments, acute lethal toxicity tests were rated highest of 15 tests (including chemical analyses and various acute and chronic sublethal tests) by both Brungs and Mount (1978) and Macek *et al.* (1978). Criteria used in these evaluations included the following: ecological significance of effects; scientific and legal defensibility; availability of acceptable methodology; utility of test results in predicting effects in aquatic environments; general applicability to all classes of chemicals; and simplicity and cost of the test.

Basic procedures for acclimating test fish to laboratory conditions and for conducting fish bioassays should be according to provincial and federal guidelines (Environment Canada 1980; B.C. Ministry of Environment 1982), as well as those recommended in Standard Methods for the Examination of Water and Wastewater (APHA *et al.* 1985). Those for conducting LC50 tests with *Daphnia magna* (Table 12) should be according to provincial (B.C. Ministry of Environment 1988a) procedures. Glass (aquaria and beakers) should be used for containing test solutions wherever possible, to minimize possible differences due to adsorption of herbicide onto test vessels made of other substances.

Emphasis should be placed on testing formulations (end-use products) rather than active ingredients, because these represent the substances entering receiving waters.

3.2.2 Tests for chemical stability in water

Certain herbicides may be volatile or may hydrolyze rapidly to a less toxic form upon mixing with water (e.g., ester vs. acid forms of 2,4-D and triclopyr) (Finlayson and Verrue 1985; Servizi *et al.* 1987). Others may increase in toxicity due to the formation of more toxic degradation or hydrolysis products (Mayer and Ellersieck 1986). The degree to which prospective new herbicides are unstable upon introduction to water can best be realized by conducting comparative 96-h LC50 tests under static versus flow-through conditions (Table 12). It is recommended that the rate of solution exchange in flow-through tests be at least once every 6 h. Each test should be duplicated, and bioassay conditions should be identical.

Geometric means of the LC50 values derived from duplicate tests should be calculated, and the ratio of static to flow-through test results determined. The classification adopted by Mayer and Ellersieck (1986) while undertaking 123 paired tests with pesticides (mainly insecticides) is recommended, as follows:

ratio <0.5: herbicide classified as more toxic under static than under flow-through conditions;

ratio 0.5-1.5: no difference in toxicity;

ratio >1.5: herbicide more toxic under flow-through than under static conditions.

Assuming acceptable analytical procedures are available, chemical concentrations of the active ingredient (and of the hydrolyzed or degraded chemical, if known) should be determined for samples of test solutions removed from the test aquaria at 0, 24, 72, and 96 h, and extracted or preserved (using accepted procedures) until analysed.

Chemical deactivation during prolonged contact with freshwater should also be tested. This provides a measure of the degree to which the herbicide degrades when dispersed in oxygenated freshwater, and of the effects of this degradation or chemical conversion on resulting toxicity. The test procedure recommended (Mayer and Ellersieck 1986) requires duplicate test solutions with a range of concentrations to be prepared. One set of solutions should be prepared initially, and held in separate glass aquaria (no fish present) for 7 days (Mayer and Ellersieck 1986) under aeration, lighting, and temperature conditions used in the bioassay test (B.C. Ministry of Environment 1982). Thereafter, a second set of fresh solutions (same concentrations) is prepared, and fish (Table 12) from the same population and stock tank placed in each (both aged and fresh solutions). A 96-h LC50 value (static test) should be determined for each set of solutions (aged and fresh), under identical test conditions.

Deactivation indices (Marking and Dawson 1972) should be calculated by dividing the 96-h LC50 value for the aged test solutions by the 96-h LC50 for the fresh solutions. The ability of the test herbicide to deactivate (detoxify) during prolonged contact with water (under bioassay test conditions) should be classified as follows (Mayer and Ellersieck 1986):

ratio <0.5: herbicide more toxic after aging in water;

ratio 0.5-1.5: aged and fresh solutions equally toxic;

ratio >1.5: aged solutions less toxic.

3.2.3 Comparative tests of time/concentration effects

Information on the dynamics of herbicide strengths within forest streams following spraying applications, indicates that a concentration "spike" may occur within the initial few hours after treatment, particularly when direct overspraying or aerial drift has occurred (see Section 2.5). These spikes dissipate rapidly and, in most instances (at least in flowing streams), reach very low (<10 µg/L) levels within 24 h post-spray. As well, detectable herbicide concentrations in forest streams are frequently evident for a few hours following the initial rain event(s) post-spray (even where buffer zones are employed). However, the magnitude of these elevations is appreciably less than that found initially due to direct overhead spraying. The concentration and persistence of herbicides in forest ponds, sloughs, and lakes after treatment is largely unknown, as little monitoring of these waterbodies has been undertaken.

A series of acute lethal tests with selected salmonid fish and freshwater invertebrate species, whereby the toxic impact of various brief (1, 2, 6, 24, 48, 96 h) exposures is tested and compared, is proposed as part of the present protocol for evaluating the toxic risk of forestry herbicides (Table 12). The results of these tests will provide an understanding of the acute lethal and observable sublethal effects (see Section 3.3.1) caused by each of these time/concentration relationships, for each of the test organisms employed. A comparison of data derived from these studies, together with field data of herbicide dynamics in forest waterbodies following spray operations, will enable a more meaningful assessment of the degree of acute threat posed by each herbicide to exposed aquatic life.

The following organisms are proposed as appropriate test species for time/concentration effect evaluations of forestry herbicides:

rainbow trout (*Salmo gairdneri*)

coho salmon (*Oncorhynchus kisutch*)

daphnids (*Daphnia magna*)

Rainbow trout and daphnids are selected, since comparative studies with 63 freshwater fish and invertebrate species tested against 174 pesticides showed these organisms to be amongst the most sensitive (Mayer and Ellersieck 1986). As well, both species are readily cultured and available year-round for testing; researchers are familiar with performing toxicity tests with them; and they are consistent with the intent of this protocol. Coho salmon are included to assure the protection of the commercial salmon resources. This species overwinters year-round in shallow, slow-moving forest streams. These waterbodies may be more susceptible to overspray because of their small size; and would likely have low dilution and flushing rates. Thus, coho are the Pacific salmon species most likely to be exposed to accidental drift or overspray. Other Pacific salmon species (e.g., sockeye or chinook salmon) could be included or substituted, depending on availability and regional concerns for their exposure.

As judged by the species-comparative data of Mayer and Ellersieck (1986) and earlier investigators, three to five aquatic species, if selected from amongst the more sensitive freshwater organisms, should be adequate for estimating freshwater animal sensitivity to herbicides and other aquatic contaminants. These researchers chose rainbow trout, *D. magna*, amphipods (*Gammarus sp.*), and in many instances a species of stoneflies or mayflies. Tests with amphipods and mayflies or stoneflies are not included in this protocol, as regional experience with culturing and conducting toxicity tests using these organisms has yet to be developed. Appropriate methodologies for the culturing of amphipods (*G. lacustris*) and mayflies (*Hexagenia sp.*) have been published by Federal Department of Fisheries scientists at the Freshwater Institute, Winnipeg, Man. (de March 1981; Friesen 1981).

The experimental evaluation of *Gammarus sp.* and *Hexagenia sp.* for time/concentration effects of prospective or current-use forestry herbicides, in conjunction with similar tests with rainbow trout, coho salmon and *D. magna*, is recommended. Static bioassays with amphipods are proposed, whereby test organisms are held in a range of herbicide strengths for periods of 1, 2, 6, or 24 h, and transferred to freshwater for observations of post-exposure survival (total test duration, 96 h). Results are compared to concurrent bioassays with amphipods, where herbicide exposures are maintained for 96 h (Table 13). Acute toxicity tests with mayfly or stonefly nymphs should employ a flow-through apparatus (Poirier and Surgeoner 1988). Test exposures of 1, 2, 6, and 24 h, with periods of recovery in freshwater to 48 h (total test period), are suggested (Table 13).

3.2.4 Tests for temperature effects

Variations in water temperature by 10°C or less can cause 5-fold differences in LC50 values derived for salmonid fish exposed to forestry herbicides (see Section 2.2.7). Ten-fold or greater differences in aquatic toxicity due to temperature have been found for a number of insecticides (Mayer and Ellersieck 1986). These differences depend on a number of factors, including the nature of the test chemical, its bioavailability, and its rate of uptake and metabolism by the exposed organism. Site-to-site and seasonal (spring to late summer) variations associated with the application of herbicides in British Columbia forests will undoubtedly result in differences of receiving water temperature by at least 10°C.

This bioassay protocol includes tests designed to determine and compare the degree to which the toxicity of prospective or in-use herbicides is altered by test (water) temperature. Temperature effect bioassays are proposed using both rainbow trout and *Daphnia magna* as test organisms (Table 12). Coho salmon and/or other Pacific salmon species could also be included in temperature effect bioassays if results with rainbow trout show a significant modifying influence on toxicity due to temperature. For simplicity, these tests should be static bioassays and the test period of conventional duration (96 h for fish; 48 h for daphnids). To standardize and simplify this procedure further, and to minimize the degree of temperature shock imposed on the test organisms when introduced to each herbicide solution, it is recommended that both stock populations of fish and daphnids be properly acclimated (B.C. Ministry of Environment 1982; American Public Health Association *et al.* 1985) to 15 ± 1°C prior to testing.

Separate 48-h (with *D. magna*) or 96-h (with rainbow trout) LC50 bioassays are to be performed with each test species at the following test temperatures: 10 ± 1°C, 15 ± 1°C, and 20 ± 1°C. For each species, organisms acclimated to 15 ± 1°C are to be tested concurrently at each of these three temperatures. Duplicate LC50 tests should be conducted at each temperature for each test organism.

A geometric mean for each paired comparison should be calculated, and the ratio of the highest to lowest mean LC50 values determined for each species (Mayer and Ellersieck 1986). This ratio will be useful in comparing the magnitude of effect of a 10°C variation in temperature on acute lethal toxicity, attributable to both the test herbicide and test species evaluated.

Herbicide concentrations in select test solutions should be measured at the initiation and termination of each temperature effect bioassay. This information will help explain chemical behavior at each temperature, as well as the extent to which alterations in chemical bioavailability due to temperature contributed to the results observed.

3.2.5 Tests for pH and receiving-water effects

As indicated previously (Section 2.2.7), the pH of the diluent (receiving) water can markedly influence the aquatic toxic impact of forestry herbicides. The degree and nature of this modifying influence is chemical-specific.

A test for pH effect on herbicide toxicity is included in the proposed protocol (Table 12). Rainbow trout are the recommended test species. Fish should be small (swimup fry) to minimize the required volume of test solutions.

For this test, separate 96-h LC50 bioassays are to be performed at each of several pH values, ranging from approximately 6.4 to 8.0. These pH values are chosen to encompass the normal range of pH for British Columbia surface waters, and to address the practicalities of maintaining test solutions at a range of pH values similar to those spanned by typical receiving waters. Required volumes of four separate diluent waters, ranging in pH from 6.4 to 8.0 (Table 12), are to be prepared using reagent grade salts ($NaHCO_3$, $CaSO_4.2H_2O$, $MgSO_4$, KCl) according to the formula prescribed by Peltier and Weber (1985). De-ionized water may be used for preparing these waters; uncontaminated surface water used routinely by the test laboratory may also prove satisfactory if it is sufficiently soft (e.g., Vancouver dechlorinated tap water). The use of other chemicals (e.g., KH_2PO_4, NaOH, H_3BO_3) for buffering pH (ASTM 1980) is not recommended because the reaction of certain herbicides with these chemicals may produce spurious results (Mayer and Ellersieck 1986).

The toxicity of the herbicide being investigated at each pH range should be determined by duplicate bioassays. The same fish stock should be used for each test, and all comparative tests should be performed concurrently where possible. Aeration of each herbicide solution should be minimal. Test pH should be monitored hourly during the first 4 h of the test, and at 24, 48, 72, and 96 h (test completion).

The geometric mean 96-h LC50 value derived for each paired comparison (duplicate tests using same diluent water) should be calculated, and the ratio of the highest to lowest LC50 determined. Correlations between average test pH and LC50 values derived for each diluent water should also be calculated.

Depending on the nature of the chemical and its speciation when mixing with surface waters of differing quality (in terms of various pH values, and differing dissolved, colloidal, and suspended organic and in organic constituents), the toxic impact of a particular herbicide within receiving waters may differ markedly (see Section 2.2.7). A series of comparative LC50 bioassays is proposed, using a number of natural, uncontaminated British Columbia surface waters as the diluent water in each test. This will ascertain the extent to which the toxicity of the herbicide under evaluation may be altered because of receiving water quality.

Before this test is undertaken, records of water quality for lakes, ponds, rivers, and streams in British Columbia forests (available from the Ministry of Environment, Environment Canada, and Fisheries and Oceans Canada) should be reviewed to identify a number of natural, uncontaminated sources. These should differ appreciably in pH and other characteristics, such as humic and fulvic acid content, total organic carbon content, and suspended solids content. Depending on existing data bases, some additional water quality analyses may be required as part of this selection process. A minimum of 8 - 10 natural water sources would be suitable for receiving water effect studies, and for comparative studies with all herbicides evaluated for toxic risk. Consideration should be given to site remoteness and ease with which water samples can be collected and transported (e.g., using 20-L plastic water jugs) to bioassay test laboratories.

The proposed test for receiving water effects will use hatchery-reared rainbow trout swimup fry and will be conducted as 96-h static LC50 tests (to minimize water volume requirements). Duplicate LC50 tests will be conducted using each of the 8 - 10 natural freshwater sources collected as the diluent water. Comparative LC50 tests using the water source to which the fish stock are acclimated should be included. As well, fish survival in duplicate freshwater "control" solutions (no herbicide present) should be determined, using each natural water sample as control water.

Each water source should be adjusted to the same test temperature (15°C), and aerated to saturation before the introduction of test fish. Fish survival and behavior (Section 3.3) should be monitored and recorded throughout each 96-h test.

Each test-water sample should be analysed for various characteristics, including pH, alkalinity, hardness, total organic carbon, suspended and dissolved solids, turbidity, humic and fulvic acid content, conductivity, and dissolved metal (e.g., Zn, Cu, Fe, Pb, Cd) content. Analysis of samples of test solutions taken from each bioassay at 0 and 96 h for herbicide concentration is desirable to determine the influence of receiving water chemistry on herbicide persistence and fate.

As with the pH effect tests, geometric mean LC50 values for each paired comparison should be calculated, and the ratio of highest to lowest values ascertained. This number will be useful in evaluating and comparing the extent to which receiving-water quality may influence the toxic impact of this and other forestry herbicides on exposed salmonid fish and other aquatic species. Correlation coefficients of LC50 values, versus each of the water quality characteristics measured for the natural water sources used as diluent waters, should also be calculated and examined. Significant correlations should indicate the importance of various receiving water characteristics on herbicide toxicity.

3.3 Acute Sublethal Tests

3.3.1 Overt sublethal effects

Recorded observations of overt sublethal toxic responses of rainbow trout and coho salmon should be made during the comparative evaluations of time/concentration effects of herbicide exposure on fish survival (Section 3.2.3). Terms and definitions of appropriate observations (including increased or decreased swimming activity, surfacing, loss of equilibrium, pigmentation changes, and respiratory rates increased or decreased from control fish) are listed in Table 14. The proposed durations of herbicide exposure and recovery for each of these acute sublethal time/concentration effect tests are given in Table 12.

A minimum of six *sublethal* concentrations of each herbicide evaluated should be included as part of each of these bioassays. At least one and preferably two of the lowest concentrations tested should not

cause any observed differences from the control fish, throughout the 96-h test period. Observations of overt sublethal responses (Table 14) should be made and recorded for each test solution (including those resulting in fish deaths) at hourly intervals throughout the initial 6 h; and at 24, 72, and 96 h for all surviving fish. The LOEC causing any observed differences compared to control fish should be reported for each fish species and each test (i.e., where herbicide exposures are 1, 2, 6, 24, or 96 h, with recovery periods of 95, 94, 90, 72, and 0 h, respectively).

Observations of overt sublethal effects of herbicides towards *Gammarus sp.* and *Hexagenia sp.* (or other suitable freshwater invertebrate species) should be similarly made and recorded during any acute tests for time/concentration effects conducted with these species (Table 13). Observations of changes in swimming speeds, respiration rates, and activity patterns of adult amphipods are appropriate, as such responses have been monitored previously in bioassay tests with *G. lacustris* (de March 1981). Flow-through tests (simulated stream studies) using mayflies, stoneflies, or other sensitive stream invertebrates could include monitoring for invertebrate drift (Poirier and Surgeoner 1988) as well as other overt sublethal responses during acute exposure and recovery studies (Table 13). These tests must include recorded observations for the presence or absence of behavioural changes during the recovery period in freshwater. As with the tests proposed for salmonid fish, each of these invertebrate bioassays should include a range of sublethal test concentrations, sufficient to permit a meaningful definition of the LOEC. Experimental work is required to select the most appropriate invertebrate species and to delineate the nature and types of overt sublethal responses to be monitored.

TABLE 14. Definition of fish behaviour terms[a]

Term	Definition
General behavior:	Observable responses of the test fish, individually or in groups, to their environment.
a. Quiescent:	Marked by a state of inactivity or abnormally low activity; motionless or nearly so.
b. Hyperexcitable:	Reacting to stimuli with substantially greater intensity than control fish.
c. Irritated:	Exhibiting more or less continuous hyperactivity.
d. Surfacing:	Rising and remaining unusually long at the surface.
e. Sounding:	Diving suddenly straight to the bottom; remaining unusually long at the bottom.
f. Twitching:	Moving the body or parts of the body with sudden jerky movements.
g. Tetanus:	In a state of tetany; marked by intermittent tonic spasms of the voluntary muscles.
h. Flaccid:	Lacking tone, resilience or firmness; weak and enfeebled.
i. Normal:	Unaffected by or not exposed to a particular experimental treatment; conforming to the usual behavioral characteristics of the species.
Swimming:	Progressive self-propulsion in water by coordinated movement of tail, body, fins.
a. Ceased:	Broken off or tapered off to a stop.
b. Erratic:	Characterized by lack of consistency, regularity, or uniformity; fluctuating, uneven; eccentric.
c. Gyrating:	Revolving around a central point; moving spirally about an axis.
d. Skittering:	Skimming hurriedly along the surface with rapid body movements.
e. Inverted:	Turned upside down, or approximately so.
f. On side:	Turned 90° laterally, more or less, from the normal body orientation.
Pigmentation:	Color of skin due to deposition or distribution of pigment.
a. Light discolored:	Color appearance lighter than usual for the species.
b. Dark discolored:	Color appearance darker than usual for the species.
c. Varidiscolored:	Color appearance abnormally varied; mottled.
Integument:	The skin.
a. Mucus shedding:	Observably losing mucus skin coating to an abnormal degree.
b. Mucus coagulation:	Showing observable clumping or clotting of the mucus skin coating, especially at the gills.
c. Hemorrhagic:	Visibly bleeding as from gills, eyes, anal opening.
Respiration:	Physical action of pumping water into mouth and out through gills, so as to absorb oxygen.
a. Rapid:	Observably faster than normal to a significant degree.
b. Slow:	Observably slower than normal to a significant degree.
c. Irregular:	Failing to occur at regular or normal intervals.
d. Ceased:	Broken off or tapered off to a stop.
e. Gulping air:	Swimming at surface with mouth open and laboriously pumping surface water and air through gills.
f. Laboured:	Performed with apparent abnormally great difficulty and effort.

[a] From Peltier and Weber (1985).

3.3.2 Avoidance/preference responses

A test for herbicide effects on avoidance and/or preference responses of underyearling rainbow trout and coho salmon is included as part of this protocol for evaluating the toxic threat presented by prospective or in-use forestry herbicides. The proposed test procedure uses a sharp-gradient avoidance/preference apparatus (Figure 1A) designed by Federal Fisheries scientists (Scherer and Nowak 1973). With this apparatus, reproducible data have been derived, marking the threshold concentrations of herbicides and other aquatic contaminants capable of repelling or attracting groups of underyearling salmonid fish (Gordon and McLeay 1978; McLeay and Gordon 1979, 1980b). Other avoidance/preference apparatus, including Y-shaped troughs (Figure 1B), can also be used to test fish given a choice between freshwater and dilute concentrations of herbicides or other aquatic contaminants (Hansen *et al.* 1972; Maynard and Weber 1981; Hildebrand *et al.* 1982).

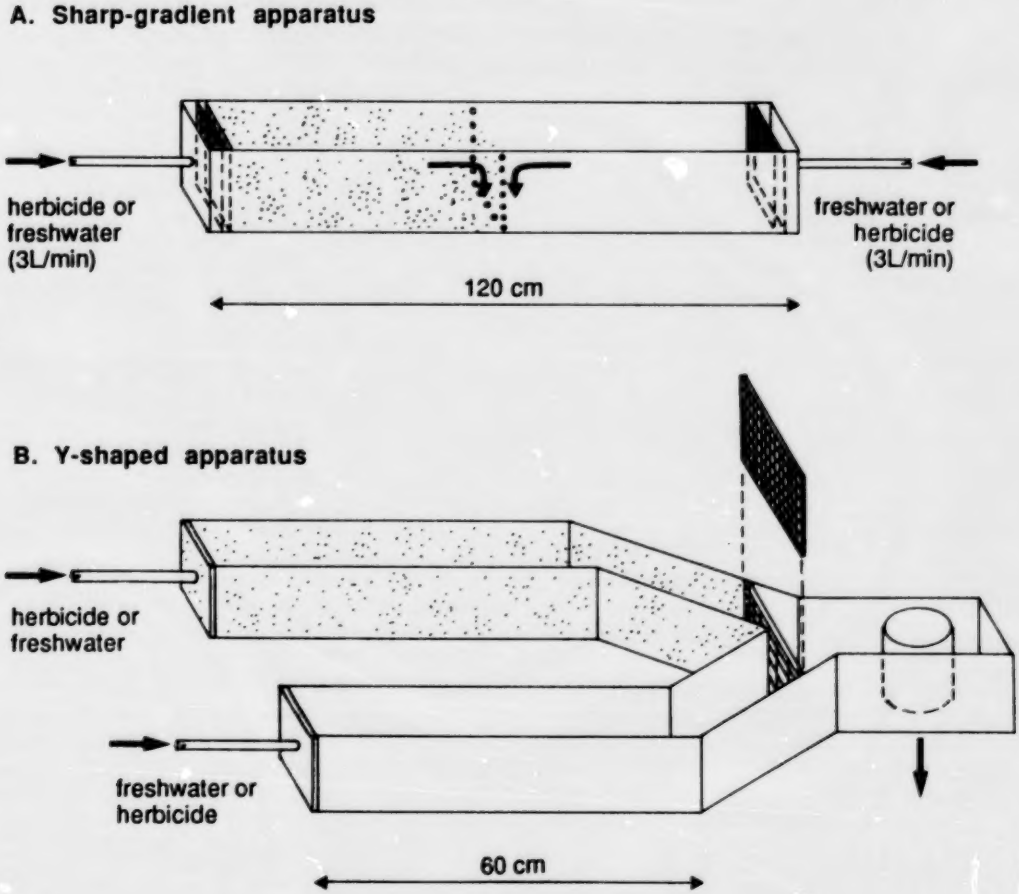

FIGURE 1. Apparatus for determining the avoidance and/or preference responses of salmonid fish to forest-use herbicides.

To do this test, a group of 10 fish is transferred to the test apparatus and allowed to acclimate for 30 min, during which time freshwater flows into each end of the trough (draining through holes in the middle). A particular sublethal herbicide concentration is then introduced to the trough through one end, with freshwater inflow (same flow rate) continuing through the other. After a further 10-min period (to allow a sharp chemical gradient establish in the trough), the number of fish in the uncontaminated freshwater side is counted and recorded at 5-min intervals throughout a 1-h test period (McLeay and Gordon 1979; Table

12). This procedure is repeated with new groups of fish and a range of concentrations for each herbicide to be tested, as well as with groups of control fish (no herbicide exposure). The trough end receiving freshwater should be alternated throughout the test to eliminate any side preference by groups of fish. Duplicate tests are recommended for each concentration evaluated.

The mean value for numbers of fish in each test solution (n = 12) should be calculated, and the geometric mean for duplicate comparisons determined. Median effective concentrations causing avoidance and/or attraction responses should also be calculated (McLeay and Gordon 1979, 1980b) for each fish species and each herbicide tested. These concentrations should be expressed as both nominal herbicide strengths (mg/L; µg/L) and as a fraction of the 96-h LC50 value (e.g., 0.05 LC50, 0.3 LC50) derived from a conventional static bioassay conducted concurrently, using the same fish stock as that used for the avoidance/preference test.

3.3.3 Acute stress tests

Tests are proposed to ascertain (under controlled laboratory conditions) the threshold concentrations of each herbicide which cause acute stress responses for exposed salmonid fish. The basic procedure recommended is one used successfully with pesticides and other aquatic contaminants (McLeay and Gordon 1979, 1980b). For each herbicide evaluated, it is recommended that stress tests be conducted using both fingerling rainbow trout and fingerling coho salmon (or an alternative Pacific salmon species) as test fish.

The proposed stress tests are both sensitive and cost-efficient, being based on secondary rather than primary (i.e., ACTH, corticosteroids, or catecholamines) physiological stress reactions (Wedemeyer *et al.* 1984, 1988). This test is conducted by transferring groups of 10 fish to separate test aquaria containing flowing freshwater only. Following a 48-h period to adapt to the transfer (handling can cause stress responses), fish in each aquarium are exposed to a particular sublethal herbicide strength. Thereafter (or following a post-exposure recovery period in freshwater), all fish are sacrificed and their blood collected for subsequent analysis of hematocrit, leucocrit (representative of white blood cell count), and plasma glucose content (McLeay 1977; McLeay and Gordon 1977).

The following exposure regime is proposed for stress tests with each herbicide and fish species (Table 12):

a) 1-h exposure, with 23-h recovery in freshwater before sampling;

b) 24-h exposure, followed by immediate sampling.

As with the avoidance/preference testing, duplicate testing of each of a range of sublethal herbicide concentrations is recommended. The plasma glucose and leucocrit values determined for individual fish exposed to each herbicide strength tested are compared to those for corresponding control groups treated identically except for the presence of herbicide. For each variable and treatment, the number of blood samples with values differing significantly from corresponding control values is calculated, and mean responses determined for each paired comparison. Median effective concentrations causing these stress responses should be calculated and expressed as both nominal herbicide strength (mg/L; µg/L) and as a fraction of the 96-h LC50 value (static test) derived concurrently, using the same fish stocks and test conditions (McLeay and Gordon 1980b). These results should be compared for differences due to fish species, duration of exposure, and recovery following brief exposure.

Additional stress-effect studies would be worthwhile to compare the sensitivity, cost, and reproducibility of these secondary stress responses with other indices of stress (e.g., plasma cortisol or catecholamine concentrations). Further work which examines the degree and rate of recovery from stress for herbicide-exposed fish is warranted, using more prolonged post-exposure periods in freshwater before sampling. This experimental work could also include studies of fish tolerance to challenge by additional environmental stressors (such as hypoxia, temperature extremes, or other aquatic contaminants), following their brief exposure to stressful sublethal concentrations of herbicides (Wedemeyer *et al.* 1984, 1988) (Table 13).

3.3.4 Salinity tolerance tests

Part of this protocol includes tests to determine how brief exposure of Pacific salmon smolts to dilute herbicide strengths, while they are in freshwater, can affect their subsequent salinity tolerance. Fully

smolted (silvered) coho salmon are recommended for these tests, although smolts of other species of Pacific salmon (such as sockeye, chinook) could be substituted or evaluated with coho.

The proposed tests for herbicide impact on subsequent salinity tolerance are based on the revised seawater challenge test developed by Blackburn and Clarke (1987). According to this procedure, groups of freshwater-acclimated smolts are transferred directly to seawater (30 o/oo salinity). After 24 h in seawater, all fish are sacrificed and their blood collected and stored until analysed for blood sodium content. Functional coho salmon smolts with good seawater growth potential are able to regulate their plasma sodium levels to 170 meq/L or less within 24 h following seawater transfer. Incompletely functional smolts, however, have higher blood sodium values (Blackburn and Clarke 1987).

For these tests, groups of salmon smolts (10 per group) should be held in a range of sublethal concentrations of each herbicide under investigation, for periods of 1 and (in separate bioassays) 24 h. Thereafter, these fish groups should be transferred to 30 ± 1 o/oo seawater, and sacrificed for blood sodium analyses after a 24-h period in seawater. Corresponding control groups should be treated identically. Additional salinity tolerance tests are proposed, in which groups of smolts are exposed to a range of dilute herbicide strengths for the same duration (1 or 24 h), and allowed a subsequent period of recovery in freshwater (95 h for 1-h exposed fish; 72 h for 24-h exposed fish) before the seawater challenge (Table 12). Duplicate testing for each of these regimes is recommended. Plasma sodium values determined for each herbicide-exposed group should be examined for significant differences relative to those for corresponding control groups. Numbers of fish showing significant differences due to each herbicide strength tested should be determined, and median effective concentrations calculated for each exposure regime evaluated. Results should be apprised with respect to EC50 values, and to durations of exposure and post- exposure recovery in freshwater before seawater challenge.

Concurrent 96-h LC50 tests (static, no freshwater recovery) should be performed with each herbicide and population of salmon smolts evaluated by salinity challenge tests. Results for each acute sublethal test should be expressed both in terms of chemical strength (mg/L; µg/L) and fraction of the LC50 value eliciting a response.

Research projects would be worthwhile if designed to investigate the relationship between these results and those from a study of the survival and growth of salmon smolts exposed briefly to sublethal strengths of herbicides that significantly increase blood sodium values. Such studies might also include a comparison of the sensitivity and cost-efficiency of other physiological approaches for testing the impact of prior herbicide exposure on seawater adaptability (e.g., elevated plasma chloride levels, increased blood osmolality) (Wedemeyer *et al.* 1988).

3.4 Chronic Sublethal Tests

3.4.1 General

Designing laboratory methods for measuring the long-term toxic threat towards regional fisheries resources, posed by the planned application of forestry herbicides, is a formidable challenge. Principal considerations include the predictive worth of such methods to real-world situations, the costs of chronic toxicity tests, and the time required for their completion. Whereas chronic testing with all life stages of sensitive invertebrate species (e.g., daphnids) can be completed within 3 weeks or less, complete life-cycle tests with salmonid fish species would require years to complete, and partial life cycle studies with early developmental stages might last several months. The cost of such lengthy tests using salmonid fish could well outweigh their potential benefit -- even without considering their inherent risks for failure due to equipment and power malfunctions, disease problems, and other unplanned events.

Besides the selection of appropriate test organisms and life stage(s) for evaluations of delayed or long-term toxic effects, decisions must be made about: variables such as continuous exposure versus dosed exposure followed by transfer to freshwater, with subsequent observations of delayed toxic effects; test type (static, static replacement or flow-through); test temperature; and biological responses monitored. No design can effectively evaluate the possible effects of each of these variables on test results and on their application to field situations, without incurring inordinately high costs. Thus, selections and compromises must be made.

The test protocol designed here for evaluating the chronic toxic threat towards regional fisheries resources, due to prospective or in-use forestry herbicides, is restricted to partial life-cycle tests with two

salmonid fish species (rainbow trout and coho salmon), and to complete life-cycle tests with *Daphnia magna*. The proposed fish tests monitor the long-term well-being of short-term herbicide exposures at various sensitive life stages; the proposed daphnid bioassay evaluates the long-term impact of continuous exposure to all life stages.

3.4.2 Tests with salmonid fish

The long-term effects of acute herbicide exposure are to be assessed by dosing the following life stages of both rainbow trout and coho salmon (or an alternative Pacific salmon species) with a range of concentrations of each herbicide under study: green (freshly fertilized) eggs; eyed eggs; alevins (yolk-sac fry); and swimup fry. Exposures of each life stage should be static (no solution replacement) and for 4 days (96 h) (Table 12). A single, 4-day exposure for each life stage is proposed (Table 12). Longer exposures of these early life stages to elevated herbicide strengths are not included because these are inconsistent with typical field-exposure conditions (see Section 2.5). Tests for effects from shorter exposures (e.g., 1 - 24 h) might also be performed where detailed information is required regarding the likely toxic impact of very brief (hours) herbicide encounters. However, in consideration of cost-effectiveness, the basic test protocol recommended for testing the susceptibility of early life stages to herbicide exposure is restricted to a 4-day exposure (Table 12) as this will provide a "worst-case" evaluation of the relative sensitivity of these versus other (e.g., fingerling, smolt) life stages.

Basic test procedures for conducting these exposures should be according to provincial guidelines (B.C. Ministry of Environment 1982). The range and number (10 - 12) of herbicide strengths to which each life stage is exposed should be selected by preliminary LC50 bioassays, and be sufficient to provide a 96-h LC50 value for each of these developmental stages as well as a minimum of five to six sublethal concentrations. A suitable range of sublethal test concentrations would be equivalent to 0.0 (control), 0.001, 0.01, 0.1, 0.3, and 0.7 of the respective 96-h LC50 value for each life stage.

A suitable apparatus for conducting tests with egg and alevin life stages is that used by Martens *et al.* (1980) and McLeay and Gordon (1979) in partial life-cycle studies with 2,4-D and Krenite®. Groups of 50 eggs or alevins are placed in separate 800-mL plastic beakers with screen mesh bottoms. A series of beakers is suspended in freshwater or the test solution within a glass aquarium; an upwelling circulation is provided by the gentle bubbling of air below the center of each mesh bottom (Martens *et al.* 1980). On completion of the 4-day (static) exposure, eggs or alevins are held in continuously running freshwater (same temperature and quality as the diluent water) until they reach the swimup fry stage, whereupon a sub-sample of 25 individuals from each of the selected treatments is transferred to flowing-water troughs or tanks for subsequent (daily) feeding and observation. Groups of fish at this stage of development are also exposed to a range of herbicide concentrations sufficient to provide a 96-h LC50 value for swimup fry. As well, 4 - 6 groups are exposed, allowing observations of post-exposure effects. Observations of each group of surviving swimup fry transferred to flowing-water troughs or tanks are continued for 28 days, and then the test is terminated.

Data end-points for these tests include:

a) *green, eyed eggs*: 96-h LC50; time to hatching; percentage hatching success; time to swimup; percentage survival to swimup; growth of swimup fry; overt behavior and appearance of swimup fry; percentage survival during and at test termination (swimup fry + 28 days).

b) *alevins:* 96-h LC50; time to swimup; percentage survival to swimup; growth, overt behavior/appearance and survival of swimup fry during 28-day period of observation as free- swimming fish.

c) *swimup fry:* 96-h LC50; growth; overt behavior and appearance; percentage survival during 28-day observation period.

The LOEC found to affect each species and life stage treated with various herbicide strengths is reported, based on the above records. These values should be expressed both in terms of mg/L (or µg/L), and as that fraction of the respective 96-h LC50 derived for each life stage examined for immediate and long-term effects of acute herbicide exposures.

Future research programs might wish to compare the sensitivity, cost efficiency, and environmental worth of this test protocol, with other laboratory test procedures now being developed and proposed for

assessing the long-term hazard of aquatic contaminants towards salmonid and other sensitive fish species (B.C. Ministry of Environment 1988b; Rexrode and Armitage 1987a). If sufficient chronic effect data indicate (by comparative studies) that one of the two test fish species examined by the present protocol (e.g., rainbow trout) is consistently as, or more, sensitive to a number of forestry herbicides tested, consideration should be given to restricting chronic toxicity tests with herbicides to a single salmonid fish species.

3.4.3 Test with *Daphnia magna*

The 21-day chronic toxicity test using *Daphnia magna* has been selected for several reasons: the test procedure is standardized (Rexrode and Armitage 1987b); the test is sensitive and relatively simple to perform at reasonable cost; and a few Canadian laboratories are already familiar with undertaking the test (Sergy 1987). Other "standardized" cladoceran life-cycle tests are available using *Ceriodaphnia sp.* (Birge and Black 1985; Horning and Weber 1985; Sergy 1987). Tests using these species can be completed within as little as 7 days when conducted at a temperature of 25°C. However, this test requires 28 days for completion when performed at 18°C (McNaught and Mount 1985) and has a number of disadvantages, including the relatively small size of *Ceriodaphnia sp.*

The 21-day life-cycle test with *D. magna* should be conducted according to the basic procedures specified recently by the U.S. Environmental Protection Agency (Rexrode and Armitage 1987b). This procedure includes the static replacement of each test solution every Monday, Wednesday, and Friday. Test temperature is 20 ± 2°C. Diluent and control water can be a suitable supply of uncontaminated surface or well water. Alternatively, reconstituted water may be used.

This test measures the effect of continuous exposure to dilute sublethal concentrations of herbicides on daphnid survival, growth, and reproduction. Appropriate data end-points for this test (Gersich *et al.* 1984; Rexrode and Armitage 1987b) are as follows:

- survival of first-generation daphnids.
- mean number of broods per daphnid.
- mean total number of young produced per daphnid.
- mean size (length) of first generation daphnids at 21 days.

A 48-h static LC50 test should be undertaken before the initiation of each chronic effect test, to define this value for the test conditions employed, and to enable a selection of appropriate test concentrations for the 21-day test. A minimum of five concentrations of each herbicide and a control (all duplicated) are to be used in this chronic test. Final results for the 21-day daphnid test should be expressed as the LOEC, both in terms of exposure concentration (mg/L; µg/L) and with respect to that fraction of the 48-h LC50 causing a significant chronic sublethal effect.

3.5 Field Tests for Toxic Effects

3.5.1 General

For each prospective new forest-use herbicide, field toxicity tests should be conducted before registration, to confirm laboratory results. These tests should be performed in forest environments typical of regions where the herbicide might be applied. The nature of field tests designed to assess the immediate and/or long-term impact of forestry herbicides on exposed aquatic life is site- and event-dependent. Unlike laboratory studies where a list of appropriate test types, conditions, and procedures may be delineated, greater judgement on the part of the investigating field team is required to select appropriate procedures for fate and effect (including toxicity) studies associated with specific herbicide applications. Accordingly, the approaches recommended here are offered as a guideline to field investigators, rather than as a rigid field test protocol.

The proposed approach for field evaluations of the aquatic toxic impact of forestry herbicides complements the test protocol recommended for controlled testing of the acute and chronic threat to regional fisheries resources posed by these chemicals. Recommended field test organisms are hatchery-reared rainbow trout and coho salmon (or an alternative Pacific salmon species); native local populations of salmonid fish; and cultured and/or native daphnids, amphipods, mayfly nymphs, stonefly nymphs, and

other stream invertebrate populations. Field testing includes a combination of *in situ* bioassays with caged organisms, acute lethal and sublethal toxicity tests performed under field conditions, and sampling and monitoring of resident salmonid fish and stream invertebrate populations.

3.5.2 Salmonid fish tests

Caged fish studies with captive or hatchery-reared fingerling salmon and trout can provide valuable information about the presence or absence of adverse effects resulting from the application of forestry herbicides. To be successful, however, transplanted fish stocks must be properly acclimated to the natural waters of concern, before initiation of the spray program. A minimum of 2 - 3 weeks should be set aside for this, during which time fish should be fed and observed daily and water quality (temperature, pH, dissolved oxygen) monitored at each cage site (Hildebrand *et al.* 1982). Cages, constructed of sturdy, non-toxic, non-abrasive material, should assure ample space for fish; protect them from excessive water currents; and prevent their escape. Proper selection of sites for locating groups of caged fish is essential, and should be planned during earlier site visits. Control stations must be well removed from the influence of any direct overspraying or spray drift, and from inflowing surface or groundwater draining the watershed to be sprayed. These control sites should have water quality conditions (e.g., temperature, dissolved oxygen, pH, suspended solids, current velocity, depth) similar to those at the test sites. Downstream or test sites should be positioned at locations where maximum herbicide input would be anticipated. Pools and shallow (10 - 30 cm depth) side streams with quiescent waters, adjacent to or within the zone to be treated, should be included as control and test sites (assuming water quality is satisfactory).

Groups of 10 - 20 fingerling rainbow trout and/or coho salmon (or alternative Pacific salmon species) should be held in separate cages (two to three cages per species) at each site selected. Fish survival and overt behavior within each cage (Table 14) should be observed closely before spraying, and monitored hourly for at least 6 h after spraying, as well as daily for the next several days. Where studies permit, surviving fish should be observed until 2 - 3 days following the first significant post-spray rainfalls. Depending on the post-exposure duration (e.g., >7 days), feeding of caged fish may be advisable.

Fish capture/release surveys should be undertaken in the forest streams (riffles, pools), side streams, and ponds within and adjacent to the proposed spray site, 1 or more days before the planned spray event. The distribution and abundance (catch per unit effort) of local salmonid and other fish species should be identified and recorded.

Attention should be given to sites both upstream and within or downstream of the treatment area, including any control and test stations where caged fish are held. In instances where intensive, coordinated field monitoring programs are planned well in advance, seasonal pre-spray samplings of indigenous fish populations for distribution and abundance would be advisable. Similar surveys of resident fish populations (at the same locations) should be conducted within a few hours following the spray event, and repeated at least once shortly after the first significant rainfalls. Any observations of dead or distressed fish captured at each site should be recorded. Field monitoring of native fish populations should be continued in those instances where significant pre- versus post-spray differences in fish abundance and distribution are evident.

Monitoring pre- and post-spray observations of caged fish and indigenous fish populations are included as part of the proposed risk-assessment protocol (Table 12). Field evaluations could also include on-site LC50/EC50 tests, whereby a portion of the population of hatchery-reared rainbow trout and coho salmon (or other Pacific salmon species) transported to the site for caged fish studies are separately exposed to a range of concentrations of the herbicide to be sprayed. Such field bioassays would use the natural stream water as diluent water. For these tests, groups of 10 fish per species would be placed in separate herbicide solutions within glass aquaria. Each aquarium would then be positioned within a shallow portion of the stream to regulate test temperature to that of the stream. Static 96-h LC50/EC50 tests would be performed, with herbicide exposures of 1 or 24 h and fish observations of survival and behavior during post-exposure recovery in freshwater (Table 13). Results from these tests would be related to: the findings from the caged-fish tests; the measured herbicide strengths in receiving waters following spraying; and the results for laboratory tests conducted with the same herbicide using similar procedures. The acute tolerance of resident salmonid fish populations to the herbicide could be tested and compared to that of the hatchery-reared stocks, using identical test procedures with a portion of the native underyearling salmonid fish captured during the surveys for fish abundance and distribution.

Experimental field studies could also include an examination of samples of native fish populations captured pre- and post-spray for histological, biochemical, or physiological effects attributable to herbicide exposure. Various approaches (Bouck 1984; Donaldson *et al.* 1984; Lockhart and Metner 1984; Wedemeyer *et al.* 1984) offer promise as sensitive field-monitoring assays. However, little attention has been given to their development for such applications. Measurement of interrenal nuclear diameters for fish captured in the treatment area may prove a useful and sensitive means for quantifying stressful effects due to treatment (Donaldson *et al.* 1984). Field challenge/performance tests (e.g., tolerance to hypoxia) for samples of captured fish may also prove worthwhile in distinguishing any reduced stamina and environmental adaptability of fish populations due to prior herbicide exposure (Wedemeyer *et al.* 1988). It should be emphasized, however, that such approaches to field evaluations of fish condition are experimental and unproven.

An alternative approach for evaluating receiving waters for potential toxicity is to collect and transport samples of suspect waters for subsequent laboratory evaluation. Provided that researchers were prepared to receive and test these samples, acute sublethal (and lethal, if apparent) toxicity tests (Section 3.3) could be performed with separate reference (e.g., upstream) -- and potentially contaminated -- water samples. Testing might include acute stress bioassays or tests for behavioral effects, using fingerling rainbow trout or a Pacific salmon species. Consideration would need to be given beforehand to the volume of water required for each test.

3.5.3 Invertebrate tests

Caged-exposure studies with sensitive freshwater invertebrate species are useful field tests for the direct assessment of the short-term impact of forestry herbicides. Such tests have been undertaken successfully with *Daphnia magna* (Hildebrand *et al.* 1980) and with diverse species of stream invertebrates (such as *Simulium venustum, Isonychia sp., Pycnopsyche sp., Acroneuria sp., Ophiogomphus sp.*, and *Orconectes propinquus*) collected at the test site before spraying (Poirier and Surgeoner 1988).

D. magna is useful for testing quiescent waters for the introduction of toxic levels of herbicides following spraying operations. As with caged fish studies, success (high percentage survival of daphnids at "control" sites) is more likely to be assured if the daphnids are acclimated to environmental conditions (including receiving water chemistry) before the test period. The cage design and procedures employed by Hildebrand *et al.* (1980) for studies with Roundup® proved adequate, enabling high (92 - 100%) survival rates throughout the 10-day test period. Advantages of using daphnids for field testing include: the proven methodologies for culturing large numbers of test organisms at reasonable cost; ease of transporting cultures to the field; their known high sensitivity to pesticides; and their ability to survive caging for 10 days or longer, thus allowing the extended post-spray monitoring of receiving waters. Additionally, field data derived for caged daphnid bioassays can be related directly to that determined for the same herbicide under controlled laboratory conditions.

Several replicate cages (10 - 25 neonate daphnids per cage) should be transferred to each control and test site under investigation. Considerations for site selection are the same as those described in selecting locations for caged fish. Caged daphnids should be allowed at least 2 days for acclimation to waters at each site before spraying. One cage from each site should be removed and its contents examined for the percentage survival of daphnids, just before the spraying operation. A separate cage should again be removed from each site so that daphnid survival rates can be observed at a number of post-spray intervals (e.g., 1, 6, 24, and 48 h; and immediately following the first rainstorm event).

A useful corollary to these tests would be to conduct acute lethal (48-h LC50) bioassays with *D. magna* exposed to the same herbicide, using natural pond or stream water from the experimental site as the diluent and control water. These tests could conceivably be conducted on-site using a portion of the same population of daphnids held in cages. Various water samples from within or draining the region sprayed could also be collected and tested for toxicity to daphnids (48-h LC50 and/or chronic life cycle test) under controlled conditions. The small sample volume requirements for daphnid bioassays make the laboratory testing of field samples an attractive alternative.

Caged studies with sensitive stream-dwelling invertebrates (e.g., mayfly, stonefly nymphs, and amphipods) are suitable for field evaluations of significant toxicant loadings in forest streams (Table 12). As in the monitoring studies with caged fish, these organisms can be raised to the water surface and observed for survival (and perhaps behavioral effects) at frequent (hourly to daily) intervals following spraying.

Studies of stream invertebrate drift, before and after herbicide applications, may provide valuable information about the degree and duration of in-stream herbicide impact and recovery (Kreutzweiser and Kingsbury 1985; Poirier and Surgeoner 1988). These surveys can distinguish the overall effect of spraying on drifting biomass (potential food source for resident fish populations), and can identify those species of drift invertebrates most sensitive to treatment. Furthermore, the collection and analysis of stream drift data, in conjunction with caged invertebrate survival data and perhaps other on-site invertebrate toxicity data, enables an assessment of the predictive worth of these field toxicity tests (Poirier and Surgeoner 1988).

3.5.4 Herbicide concentrations in water

In all instances where proven analytical procedures are available, samples of surface waters should be collected during field toxicity studies and preserved for future herbicide analysis. Sampling stations should be in association with sites selected for caging studies or other field toxicity evaluations. Sampling frequency should be at least hourly for the initial few hours following spraying, and at 24, 48, and 96 h thereafter. Consideration should be given to taking time-integrated (composite) samples during this period. Subsequent sampling should be undertaken with declining frequency, except on the occasion of the first two rainfall events post-spray. At these times, daily or twice-daily sampling at each site would be desirable to define the magnitude and duration of herbicide concentrations within receiving waters. The stability of dilute herbicide strengths during storage (before analysis) should be confirmed using field "standards" (known concentrations of herbicides prepared with uncontaminated water) preserved and stored under conditions identical to the field samples.

The need for reliable estimates of environmental exposure concentrations is emphasized, as these data are a critical link in the interpretation of field and laboratory toxicity data, and in predicting impacts of the test herbicide at other sites.

3.6 Concluding Comments

The test protocol presented is comprised of a battery of lethal and sublethal toxicity test procedures using the sensitive early life stages of salmonid fish, as well as sensitive freshwater invertebrate species. This protocol has been designed to help researchers:

- understand the effect of herbicide hydrolysis and aging of dilute aqueous solutions on residual toxicity to freshwater aquatic life.
- appreciate the relationship between exposure time and herbicide strength on immediate and post-exposure toxic responses for trout, Pacific salmon, and their prey.
- measure the degree to which environmental variables may modify toxicity.
- estimate threshold sublethal herbicide concentrations that may cause immediate or long- term adverse effects towards exposed fish and invertebrate species.
- develop meaningful field procedures for detecting and monitoring toxic herbicide effects within receiving waters.

The tests proffered are necessarily extensive to provide sufficient data by which the toxic hazard to salmonid fish and their prey, posed by a particular herbicide, can be judged. Toxicity evaluations of prospective herbicides should be approached in tiers, starting with the appropriate acute lethal tests and proceeding to the more costly sublethal tests where merited. This would be justified in instances where acute lethal toxic effects in the environment appear to be extremely unlikely, and information is desired concerning threshold herbicide concentrations harmful to salmonid fish and freshwater invertebrates. On the other hand, the further (sublethal) evaluation of prospective herbicides shown to be highly toxic in acute lethal bioassays, and likely to pose a real threat to the short-term survival of these organisms, may prove fruitless. Field testing for those herbicides showing a high level of toxicity under controlled test conditions also may not be warranted. When carried out, field toxicity tests should, wherever possible, be combined with other studies designed to assess the efficacy of promising new herbicides, as well as herbicide dispersal and degradation.

Concurrent with the implementation of this toxicity test protocol, research efforts should begin to examine new approaches for assessing the degree of toxic risk to regional fisheries resources presented

by new or in-use forestry herbicides. As with the present protocol, tests should be sought which improve predictability in a cost-efficient manner.

4. REFERENCES

Adams, R.W. 1987. Handbook for pesticide applicators and dispensers. 5th Edition. B.C. Min. of Environ., Pesticide Control Branch, Victoria, B.C.

Adams, W.J., R.A. Kimerle, B.B. Heidolph, and P.R. Michael. 1983. Field comparison of laboratory-derived acute and chronic toxicity data. *In* Aquatic toxicology and hazard assessment: 6th Symp. W.E. Bishop, R.D. Cardwell and B.B. Heidolph, (editors). ASTM STP 802. American Soc. for Testing and Materials. Philadelphia, Pa., pp. 367-385.

Agriculture Canada, Environment Canada, Fisheries & Oceans Canada, and Health & Welfare Canada. 1984. Pesticide use and control in Canada. Ottawa, Ont.

Agriculture Canada, Environment Canada, and Fisheries & Oceans Canada. 1987. Guidelines for determining environmental chemistry and fate of pesticides. Report T-1-255. July 15, 1987. Agric. Can. Pesticides Directorate, Ottawa, Ont.

Alexander, H.C., F.M. Gersich, and M.A. Mayes. 1985. Acute toxicity of four phenoxy herbicides to aquatic organisms. Bull. Environ. Contam. Toxicol. 35 (3): 314-321.

American Public Health Association, American Water Works Association and Water Pollution Control Federation. 1985. Part 800. Toxicity test methods for aquatic organisms. *In* Standard methods for the examination of water and wastewater. 16th ed. Washington, D.C., pp. 689-823.

ASTM. 1980. Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians. Am. Soc. for Testing and Materials. Annual Book of ASTM Standards, E 729-80. Philadelphia, Pa.

Bathe, R., K. Sachsse, L. Ullmann, W.D. Hormann, F. Zak, and R. Hess. 1975. The evaluation of fish toxicity in the laboratory. Proc. Eur. Soc. Toxicol. 16: 113-124.

Birge, W.J. and J.A. Black. 1985. Research needs for rapid assessments of chronic toxicity. *In* Aquatic toxicology and hazard assessment: 8th Symp. R.C. Bahner and D.J. Hansen (editors). ASTM STP 891. Am. Soc. for Testing and Materials. Philadelphia, Pa., pp. 51-60.

Blackburn, J. and W.C. Clarke. 1987. Revised procedure for the 24 hour seawater challenge test to measure seawater adaptability of juvenile salmonids. Can. Tech. Rep. Fish. Aquat. Sci. No. 1515. Nanaimo, B.C.

Blaise, C. 1984. Introduction to ecotoxicological concepts. *In* Biological testing and hazard assessment. R. Van Coillie, N. Bermingham, and C. Blaise (editors). Proc. Conf. October 20-21, 1982. Environ. Can., Montreal, Que., pp. 11-47.

Bouck, G.R. 1984. Physiological responses of fish: problems and progress toward use in environmental monitoring. *In* Contaminant effects on fisheries. Vol. 16. V.W. Cairns, P.V. Hodson, and J.O. Nriagu (editors). Wiley Series in Advances in Environmental Science and Technology. Toronto, Ont., pp. 61-71.

Bouck, G.R. and D.A. Johnson. 1979. Medication inhibits tolerance to sea water in coho salmon smolts. Trans. Am. Fish. Soc. 108 (1): 63-66.

B.C. Ministry of Environment. 1982. Provincial guidelines and laboratory procedures for measuring acute lethal toxicity of liquid effluents to fish. November 1982. Victoria, B.C.

___. 1988a. Daphnia 48-hr acute mortality bioassay procedure. Environ. Lab., Vancouver, B.C.

___. 1988b. A bioassay procedure for the measurement of toxicants to eyed salmonid eggs. Environ. Lab., Vancouver, B.C.

Brungs, W.A. and D.I. Mount. 1978. Introduction to a discussion of the use of aquatic toxicity tests for evaluation of the effects of toxic substances. *In* Estimating the hazard of chemical substances to

aquatic life. J.Cairns, Jr., K.L. Dickson, and A.W. Maki (editors). ASTM STP 657. Am. Soc. for Testing and Materials. Philadelphia, Pa., pp. 15-26.

Chang, J.C., P.B. Taylor, and F.R. Leach. 1981. Use of the Microtox assay system for environmental samples. Bull. Environ. Contam. Toxicol. 26: 150-156.

Chapman, G.A. 1983. Do organisms in laboratory tests respond like organisms in nature? *In* Aquatic toxicology and hazard assessment: 6th Symp. W.E. Bishop, R.D. Cardwell and B.B. Heidolph (editors). ASTM STP 802. Am. Soc. for Testing and Materials. Philadelphia, Pa., pp. 315-327.

Clarke, W.C. and J. Blackburn. 1977. A seawater challenge test to measure smolting of juvenile salmon. Fish. Marine Serv. Tech. Rep. 705. Fish. Oceans Can. Nanaimo, B.C.

Cunningham, V.L., M.S. Morgan, and R.E. Hannah. 1986. Effect of natural water source on the toxicity of chemicals to aquatic microorganisms. *In* Aquatic toxicology and environmental fate: 9th Vol. T.M. Poston and R. Purdy (editors). ASTM STP 921. Am. Soc. for Testing and Materials. Philadelphia, Pa., pp. 436-449.

Dewey, S.L. 1986. Effects of the herbicide atrazine on aquatic insect community structure and emergence. Ecology 67 (1): 148-162.

de March, B.G.E. 1981. *Gammarus lacustris lacustris* G.O. Sars. *In* Manual for the culture of selected freshwater invertebrates. S.G. Lawrence (editor). Can. Spec. Publ. Fish. Aquat. Sci. No. 54. Fish. Oceans Can. Ottawa, Ont., pp.79-94.

DeZwart, D. and W. Sloof. 1983. The Microtox as an alternative assay in the acute toxicity assessment of water pollutants. Aqua. Toxicol. 4: 129-138.

Dodson, J.J. and C.I. Mayfield. 1979. Dynamics and behavioral toxicology of Aqua-Kleen SUP-R (2,4-D butoxyethanol ester) as revealed by the modification of rheotropism in rainbow trout. Trans. Am. Fish. Soc. 108: 632-640.

Doe, K.G., W.R. Ernst, W.R. Parker, G. Julien, and P. Hennigar. 1986. The influence of pH on the acute lethality of fenitrothion, 2,4-D and aminocarb and some pH altered sublethal effects of aminocarb to rainbow trout. *In* Proc. 11th Ann. Aquat. Tox. Workshop. G.H. Geen and K.L. Woodward (editors). Can. Tech. Rep. Fish. Aquat. Sci. No. 1480. Fish. Oceans Can. Ottawa, Ont., pp. 198-199.

Donaldson, E.M., U.H.M. Fagerlund, and J.R. McBride. 1984. Aspects of the endocrine response to pollutants in salmonids. *In* Contaminant effects on fisheries. Vol. 16. V.W. Cairns, P.V. Hodson and J.O. Nriagu (editors). Wiley Series in Advances in Environmental Science and Technology. Toronto, Ont., pp. 213-221.

Dutka, B.J. and K.K. Kwan. 1981. Comparison of three microbial toxicity screening tests with the Microtox test. Bull. Environ. Contam. Toxicol. 27: 753-757.

Environment Canada. 1980. Standard procedure for testing the acute lethality of liquid effluents. Report No. EPS 1-WP-80-1. Water Pollution Control Directorate, Ottawa, Ont..

Feng, J.C., D.G. Thompson, and P.E. Reynolds. 1987. Fate of glyphosate in a Canadian forest stream ecosystem. Paper presented at the Carnation Creek Herbicide Workshop. December 8, 1987. Nanaimo, B.C.

Finlayson, B.J. and G.A. Faggella. 1986. Comparison of laboratory and field observations of fish exposed to the herbicides Molinate and Thiobencarb. Trans. Am. Fish. Soc. 115 (6): 882-890.

Finlayson, B.J. and K.M. Verrue. 1985. Toxicities of butoxyethanol ester and propylene glycol butyl ether ester formulations of 2,4-dichlorphenoxy acetic acid (2,4-D) to juvenile salmonids. Arch. Environ. Contam. Toxicol. 14 (2): 153-160.

Fitzmayer, K.M., J.G. Geiger, and M.J. Van den Avyle. 1982. Effects of chronic exposure to simazine on the cladoceran, *Daphnia pulex*. Arch. Environ. Contam. Toxicol. 11 (5): 603-609.

Folmar, L.C. 1976. Overt avoidance reaction of rainbow trout fry to nine herbicides. Bull. Environ. Contam. Toxicol. 15 (5): 509-514.

___. 1978. Avoidance chamber responses of mayfly nymphs exposed to eight herbicides. Bull. Environ. Contam. Toxicol. 19 (3): 312-318.

Folmar, L.C., H.O. Sanders, and A.M. Julin. 1979. Toxicity of the herbicide glyphosate and several of its formulations to fish and aquatic invertebrates. Arch. Environ. Contam. Toxicol. 8 (3): 269-278.

Francis, B.M., R.L. Lampman, and R.L. Metcalf. 1985. Model ecosystem studies of the environmental fate of five herbicides used in conservation tillage. Arch. Environ. Contam. Toxicol. 14 (6): 693-704.

Friesen, M.K. 1981. *Hexagenia rigida* (McDunnough). *In* Manual for the culture of selected freshwater invertebrates. S.G. Lawrence (editor). Can. Spec. Publ. Fish. Aquat. Sci. No. 54. Fish. Oceans Can. Ottawa, Ont., pp. 127-142.

Gersich, F.M., D.L. Hopkins, and D.P. Milazzo. 1985. Acute and chronic toxicity of technical picloram (4-amino-3,5,6-trichloropicolinic acid) to *Daphnia magna* Straus. Bull. Environ. Contam. Toxicol. 35 (1): 121-126.

Gersich, F.M. and M.A. Mayes. 1986. Acute toxicity tests with *Daphnia magna* and *Pimephales promelas* in support of National Pollutant Discharge Elimination Permit requirements. Water Res. 20: 939-941.

Gersich, F.M., C.G. Mendoza, D.L. Hopkins, and K.M. Bodner. 1984. Acute and chronic toxicity of triclopyr triethylamine salt to *Daphnia magna* Straus. Bull. Environ. Contam. Toxicol. 32 (4): 497-502.

Giesy, J.P. 1985. Multispecies tests: research needs to assess the effects of chemicals on aquatic life. *In* Aquatic toxicology and hazard assessment: 8th Symp. R.C. Bahner, and D.J. Hansen (editors). ASTM STP 891. Am. Soc. for Testing and Materials. Philadelphia, Pa., pp. 67-77.

Gordon, M.R. and D.J. McLeay. 1978. Avoidance reactions of salmonids to pulpmill effluents. B.C. Research Rep. No. 688. Prepared for the Committee on Pollution Abatement Research (CPAR). Environ. Can. Ottawa, Ont.

Hamelink, J.L., D.R. Buckler, F.L. Mayer, D.U. Palawski, and H.O. Sanders. 1986. Toxicity of fluridone to aquatic invertebrates and fish. Environ. Toxicol. Chem. 5 (1): 87- 94.

Hansen, S.R. and R.R. Garton. 1982. Ability of standard toxicity tests to predict the effects of the insecticide diflubenzuron on laboratory stream communities. Can. J. Fish. Aquat. Sci. 39: 1273-1288.

Hansen, D.J., E. Matthews, S.L. Nall, and D.P. Dunes. 1972. Avoidance of pesticides by untrained mosquitofish, *Gambusia affinis*. Bull. Environ. Contam. Toxicol. 8: 46.

Hartman, W.A. and D.B. Martin. 1984. Effect of suspended bentonite clay on the acute toxicity of glyphosate to *Daphnia pulex* and *Lemna minor*. Bull. Environ. Contam. Toxicol. 33 (3): 355-361.

___. 1985. Effects of four agricultural pesticides on *Daphnia pulex, Lemna minor,* and *Potamogeton pectinatus*. Bull. Environ. Contam. Toxicol. 35 (5): 646-651.

Hattula, M.L., H. Reunanen, and A.U. Arstila. 1978. The toxicity of MCPA to fish. Light and electron microscopy and the chemical analysis of the tissue. Bull. Environ. Contam. Toxicol. 19 (4): 465-470.

Henigman, J.F. 1984. Herbicide Use in B.C. Forestry. Internal Rep. presented to the B.C. Ad Hoc Pesticide Committee on Forest Pesticides Panel Discussion on Herbicides, October 23, 1984, Vancouver. B.C. Min. For., Protection Branch. Victoria, B.C.

Hildebrand, L.D., D.S. Sullivan, and T.P. Sullivan. 1980. Effects of Roundup herbicide on populations of *Daphnia magna* in a forest pond. Bull. Environ. Contam. Toxicol. 25 (3): 353-357.

___. 1982. Experimental studies of rainbow trout populations exposed to field applications of Roundup herbicide. Arch. Environ. Contam. Toxicol. 11 (1): 93-98.

Holtby, L.B. and S.J. Baillie. 1987. Effects of the herbicide "Roundup" on coho salmon fingerlings in an over-sprayed side-channel of Carnation Creek, British Columbia. Paper presented at the Carnation Creek Herbicide Workshop. December 9, 1987. Nanaimo, B.C.

Horning, W.B. and C.I. Weber. 1985. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. Report EPA/600/4-85/014. U.S. Environ. Protection Agency. Cincinnati, Ohio.

Humphreys, P. 1987. Pesticide use in British Columbia forestry 1986. For. Serv. Internal Rep. PM-PB-28. June 1987. B.C. Min. For., Protection Branch. Victoria, B.C.

Inglis, A. and E.L. Davis. 1972. Effects of water hardness on the toxicity of several organic and inorganic herbicides to fish. U.S. Bur. Sport Fish Wildl. Tech. Pap. 67: 1-22.

Isensee, A.R. 1980. Laboratory microecosystems. *In* Hand. Environ. Chem. Vol.2 Part A. O. Hutzinger (editor). Springer-Verlag, Berlin, pp. 231-245.

Kersting, K. and H. van der Honing. 1981. Effect of the herbicide Dichlobenil on the feeding and filtering rate of *Daphnia magna*. Verhandlungen Int. Verein. Theor. Angew. Limnol. 21 (2): 1135-1140.

Kreutzweiser, D.P. and P.D. Kingsbury. 1985. Drift responses of stream invertebrates to a glyphosate application. File Rep. 63. January 1985. For. Pest Manage. Inst., Can. For. Serv.

Lawrence, S.G. 1981. Manual for the culture of selected freshwater invertebrates. Can. Spec. Publ. Fish. Aquat. Sci. No. 54. Fish. and Oceans Can. Ottawa, Ont.

Liguori, V.M., H.R. Zakour, M.L. Landolt, and S.P. Felton. 1983. Toxicity of the herbicide endothall to juvenile chinook salmon (*Oncorhynchus tshawytscha*). *In* Aquatic toxicology and hazard assessment: 6th Symp. W.E. Bishop, R.D. Cardwell and B.B. Heidolph (editors). ASTM STP 802. Am. Soc. for Testing and Materials. Philadelphia, Pa., pp. 530-544.

Lockhart, W.L. and D.A. Metner. 1984. Fish serum chemistry as a pathology tool. *In* Contaminant effects on fisheries. Vol. 16. V.W. Cairns, P.V. Hodson and J.O. Nriagu (editors). Wiley Series in Advances in Environmental Science and Technology. Toronto, Ont., pp. 73-85.

Lorz, H.W., S.W. Glenn, R.H. Williams, C.M. Kunkel, and L.A. Norris. 1979. Effects of selected herbicides on smolting of coho salmon. U.S. Environ. Protection Agency. Rep. EPA/600/3-79/071. Corvallis, Oreg.

McBride, J.R., H.M. Dye, and E.M. Donaldson. 1981. Stress response of juvenile sockeye salmon (*Oncorhynchus nerka*) to the butoxyethanol ester of 2,4-dichlorophenoxyacetic acid. Bull. Environ. Contam. Toxicol. 27: 877-884.

McLean, M.P., R.E. McNicol, and E. Scherer. 1980. Bibliography of toxicity test methods for the aquatic environment. Can. Spec. Publ. Fish. Aquat. Sci. 50. Dep. Fish. Oceans. Winnipeg, Man.

McLeay, D.J. 1977. Development of a blood sugar bioassay for rapidly measuring stressful levels of pulpmill effluent to salmonid fish. J. Fish. Res. Board Can. 34: 477-485.

McLeay, D.J. and M.R. Gordon. 1977. Leucocrit: a simple hematological technique for measuring acute stress in salmonid fish, including stressful concentrations of pulpmill effluent. J. Fish. Res. Board Can. 34: 2164-2175.

___. 1979. Concentrations of Krenite causing acute lethal, avoidance and stress responses with salmon. B.C. Research Project Rep. No. 1-04-156. Prepared for Dupont Canada Inc., Vancouver, B.C.

___. 1980a. Toxicity studies with the brush-control herbicide Krenite and salmonid fish. B.C. Research Project Rep. No. 1-01-305. Prepared for B.C. Min. For., Vancouver, B.C.

___. 1980b. Short-term sublethal toxicity tests to assess safe levels of environmental contaminants. B.C. Research Rep. No. 1-11-299. Environ. Can. Ottawa, Ont.

McNaught, D.C. and D.I. Mount. 1985. Appropriate durations and measures for *Ceriodaphnia* toxicity tests. *In* Aquatic toxicology and hazard assessment: 8th Symp. R.C. Bahner, and D.J. Hansen (editors). ASTM STP 891. American Society for Testing and Materials. Philadelphia, Pa., pp. 375-381.

Macek, K., W. Birge, F.L. Mayer, A.L. Buikema, Jr., and A.W. Maki. 1978. Discussion session synopsis - use of aquatic toxicity tests for evaluation of the effects of toxic substances. *In* Estimating the hazard of chemical substances to aquatic life. J. Cairns, K.L. Dickson, and A.W. Maki (editors). ASTM STP 657. Am. Soc. for Testing and Materials. Philadelphia, Pa., pp. 27-32.

Macek, K.J., M.A. Lindberg, S.Sauter, K.S. Buxton, and P.A. Costa. 1976. Acute and chronic toxicity of acrolein, heptachlor, endosulfan, and trifluralin to the water flea (*Daphnia magna*) and the fathead minnow (*Pimephales promelas*). U.S. Environ. Protection Agency. Rep. EPA/600/3-76/099. Duluth, Minn.

Madhun, Y.A., J.L. Young, and V.H. Freed. 1986. Binding of herbicides by water-soluble organic materials from soil. J. Environ. Qual. 15: 64-68.

Marking, L.L. and V.K. Dawson. 1972. The half-life of biological activity of antimycin determined by fish bioassay. Trans. Am. Fish. Soc. 101: 100-105.

Marking, L.L. and C.R. Walker. 1973. The use of fish bioassays to determine the rate of deactivation of pesticides. *In* Bioassay techniques and environmental chemistry. G.E. Glass (editor). Ann Arbor Sci. Publ. Inc. Ann Arbor, Mich., pp. 357-366.

Martens, D.W., R.W. Gordon, and J.A. Servizi. 1980. Toxicity of butoxyethyl ester of 2,4-D to selected salmon and trout. International Pacific Salmon Fisheries Commission. Progress Rep. No. 40. New Westminster, B.C.

Mayack, D.T., P.B. Bush, D.G. Neary, and J.E. Douglass. 1982. Impact of hexazinone on invertebrates after application to forested watersheds. Arch. Environ. Contam. Toxicol. 11: 209-217.

Mayer, F.L. and M.R. Ellersieck. 1986. Manual of acute toxicity: interpretation and data base for 410 chemicals and 66 species of freshwater animals. Resource Publ. PUB- 160. NTIS PB86-239878. Fish Wildl. Serv. Washington, D.C.

Mayes, M.A. and D.C. Dill. 1984. The acute toxicity of picloram, picloram potassium salt, and picloram tri-iso propanolamine salt to aquatic organisms. Environ. Toxicol. Chem. 3 (2): 263-270.

Mayes, M.A., D.L. Hopkins, and D.C. Dill. 1987. Toxicity of picloram (4-amino-3,5,6-trichloropicolinic acid) to life stages of the rainbow trout. Bull. Environ. Contam. Toxicol. 38 (4): 653-660.

Mayes, M.A. and G.R. Oliver. 1985. An aquatic hazard assessment: picloram. *In* Aquatic toxicology and hazard assessment. 8th Symp. R.C. Bahner and D.J. Hansen (editors). ASTM STP 891. Am. Soc. for Testing and Materials. Philadelphia, Pa., pp. 253-269.

Maynard, D.J. and D.D. Weber. 1981. Avoidance reactions of juvenile coho salmon (*Oncorhynchus kisutch*) to monocyclic aromatics. Can. J. Fish. Aquat. Sci. 38: 772-778.

Meehan, W.R., L.A. Norris, and H.S. Sears. 1974. Toxicity of various formulations of 2,4-D to salmonids in Southeast Alaska. J. Fish. Res. Board Can. 31 (4): 480-485.

Mitchell, D.G., P.M. Chapman, and T.J. Long. 1986. Acute toxicity of the glyphosate herbicide Roundup (Vision) to rainbow trout, chinook and coho salmon. Report prepared for Monsanto Co., St. Louis, Mo., by E.V.S. Consultants, Vancouver, B.C.

___. 1987. Seawater challenge testing of coho salmon smolts following exposure to Roundup herbicide. Environ. Toxicol. Chem. 6: 875-878.

Mount, D.I., N.A. Thomas, T.J. Norberg, M.T. Barbour, T.H. Roush, and W.F. Brandes. 1984. Effluent and ambient toxicity testing and instream community response on the Ottawa River, Lima, Ohio. Rep. EPA-600/3-84-080. U.S. Environ. Protection Agency. Duluth, Minn.

Muirhead-Thomson, R.C. 1987. Pesticide impact on stream fauna with special reference to macroinvertebrates. Cambridge Univ. Press., New York, N.Y.

Murty, A.S. 1986a. Toxicity of pesticides to fish. Vol. I. CRC Press, Inc., Boca Raton, Fla., 192 p.

___. 1986b. Toxicity of pesticides to fish. Vol. II. CRC Press, Inc., Boca Raton, Fla., 160 p.

Neuhold, J.M. 1986. Toward a meaningful interaction between ecology and aquatic toxicology. *In* Aquatic toxicology and environmental fate: 9th Vol. T.M. Poston and R. Purdy (editors). ASTM STP 921. Am. Soc. for Testing and Materials. Philadelphia, Pa., pp. 11-21.

Newton, M., K.M. Howard, B.R. Kelpsas, R. Danhaus, C.M. Lottman, and S. Dubelman. 1984. Fate of glyphosate in an Oregon forest ecosystem. J. Agric. Food Chem. 32 (5): 1144-1151.

Norris, L.A. 1981. The movement, persistence, and fate of the phenoxy herbicides and TCDD in the forest. *In* Residue reviews. Vol. 20. Springer-Verlag, New York, N.Y., pp. 65-135.

Norris, L.A., H.W. Lorz, and S.V. Gregory. 1983. Influence of forest and rangeland management on anadromous fish habitat in Western North America. Forest chemicals. Gen. Tech. Rep. PNW-149. Pac. NW For. Range Exp. Stn., For. Serv., U.S. Dep. of Agric., Portland, Oreg.

Norris, L.A. and M.L. Montgomery. 1975. Dicamba residues in streams after forest spraying. Bull. Environ. Contam. Toxicol. 13: 1-8.

Norris, L.A., M.L. Montgomery, and L.E. Warren. 1987. Triclopyr persistence in Western Oregon hill pastures. Bull. Environ. Contam. Toxicol. 39: 134-141.

Norris, L.A. and D.G. Moore. 1981. Introduced chemicals and water quality. *In* Interior west watershed management. D.M. Baumgartner (editor). Proc. Symp. April 8 - 10, 1980, Spokane, Wash. Washington State Univ., Pullman, Wash., pp. 203-220.

OECD. 1984. OECD Proc. of the International Workshop on Biological Testing of Effluents (and Related Receiving Waters). September 10-14, 1984. Duluth, Minn. U.S. Environ. Protection Agency and Environ. Can. Ottawa, Ont.

Palawski, D., D.R. Buckler, and F.L. Mayer. 1983. Survival and condition of rainbow trout *Salmo gairdneri* after acute exposures to methyl parathion, tri-phenyl phosphate and S,S,S-tri-butyl phosphorotrithioate. Bull. Environ. Contam. Toxicol. 30 (5): 614-620.

Pearce, B.C. and J. McBride. 1978. A preliminary investigation of the effect of 2,4-dichlorophenoxyacetic acid (butoxy- ethanol ester) on juvenile rainbow trout. Fish. Mar. Serv. Man. Rep. No. 1478. Vancouver, B.C.

Peltier, W.H. and C.I. Weber. 1985. Methods for measuring the acute toxicity of effluents to freshwater and marine organisms. Rep. EPA/600-4-85/013. U.S. Environ. Protection Agency. Cincinnati, Ohio.

Poirier, D.G. and G.A. Surgeoner. [1988]. A field bioassay to predict the impacts of aerial applications of forestry insecticides on stream invertebrates. Can. Entomol. In press.

Presing, M. 1981. On the effects of Dikonirt (sodium salt of 2,4-dichlorophenoxy-acetic acid) on the mortality and reproduction of *Daphnia magna*. Hydrobiologia 83 (3): 511- 516.

Qureshi, A.A., K.W. Flood, S.R. Thompson, S.M. Janhurst, C.S. Inniss, and D.A. Rokosh. 1982. Comparison of a luminescent bacterial test with other bioassays for determining toxicity of pure compounds and complex effluents. *In* Aquatic toxicology and hazard assessment: 5th Conf. J.G. Pearson, R.B. Foster and W.E. Bishop (editors). ASTM STP 766. Am. Soc. for Testing and Materials. Philadelphia, Pa., pp. 179-195.

Rexrode, M. and T.M. Armitage. 1987a. Hazard evaluation division standard evaluation procedure: fish early life-stage test. Rep. PB87-209722. U.S. Environ. Protection Agency. Washington, D.C.

___. 1987b. Hazard evaluation division standard evaluation procedure: *Daphnia magna* life-cycle (21-day renewal) chronic toxicity test. Rep. PB87-209730. U.S. Environ. Protection Agency. Washington, D.C.

Ribo, J.M. and K.L.E. Kaiser. 1987. *Photobacterium phosphoreum* toxicity bioassay. I. Test procedures and applications. Toxicity Assessment 2: 305-323.

Sanders, H.O. 1970. Toxicities of some herbicides to six species of freshwater crustaceans. J. Water Poll. Control Fed. 42: 1544-1550.

Sanders, H.O. and J.B. Hunn. 1982. Toxicity, bioconcentration, and depuration of the herbicide Bolero 8EC in freshwater invertebrates and fish. Bull. Jap. Soc. Sci. Fish. 48 (8): 1139-1143.

Scherer, E. 1979. Toxicity tests for freshwater organisms. Can. Spec. Publ. Fish. Aquat. Sci. 44. Dep. Fish. Oceans. Winnipeg, Man.

Scherer, E. and S. Nowak. 1973. Apparatus for recording avoidance movements of fish. J. Fish. Res. Board Can. 30: 1594-1596.

Schober, U. and W. Lampert. 1977. Effects of sublethal concentrations of the herbicide Atrazin on growth and reproduction of *Daphnia pulex*. Bull. Environ. Contam. Toxicol. 17 (3): 269-277.

Schultz, T.W. and J.R. Kennedy. 1976. Cytotoxic effects of the herbicide 3-amino-1,2,4-triazole on *Daphnia pulex* (Crustacea: Cladocera). Biol. Bull. 151: 370-385.

Scorgie, H.R.A. and A.S. Cooke. 1979. Effects of the triazine herbicide Cyanatryn on aquatic animals. Bull. Environ. Contam. Toxicol. 22 (1-2): 135-142.

Sergy, G.A. 1987. Recommendations on aquatic biological tests and procedures for environmental protection, C&P, DOE. Environ. Can. July 1987. Edmonton, Alta.

Servizi, J.A., R.W. Gordon, and D.W. Martens. 1987. Acute toxicity of Garlon-4 and Roundup herbicides to salmon, daphnia, and trout. Bull. Environ. Contam. Toxicol. 39 (1): 15-22.

Sharom, M.S. and K.R. Solomon. 1981. Adsorption and desorption of permethrin and other pesticides on glass and plastic materials used for bioassay procedures. *In* Proc. 7th Ann. Aquat. Tox. Workshop. Can. Tech. Rep. Fish. Aquat. Sci. No. 990. Ottawa, Ont., pp. 341-344.

Sigmon, C. 1979. Oxygen consumption in *Daphnia pulex* exposed to 2,4-D or 2,4,5-T. Bull. Environ. Contam. Toxicol. 21 (6): 822-825.

Sprague, J.B. 1969. Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. Water Res. 3: 793-821.

___. 1970. Measurement of pollutant toxicity to fish. II. Utilizing and applying bioassay results. Water Res. 4: 3-32.

___. 1971. Measurement of pollutant toxicity to fish. III. Sublethal effects and "safe" concentrations. Water Res. 5: 245-266.

___ 1973. The ABC's of pollutant bioassay using fish. *In* Biological methods for the assessment of water quality. J. Cairns Jr. and K.L. Dickson (editors). ASTM STP 528. Am. Soc. Testing and Materials. Philadelphia, Pa., pp. 6-30.

Taub, F.B. 1976. Demonstration of pollution effects in aquatic microcosms. Int. J. Environ. Studies 10: 23-33.

Thebault, M.T. and F.X. Decaris. 1983. Calcium ATPase inhibition in trout *Salmo gairdneri* gill microsomes following in-vitro and in-vivo treatments with 2,4,5-T. Comp. Biochem. Physiol. 75C: 369-372.

Toews, D.A.A. and M.J. Brownlee. 1981. A handbook for fish habitat protection on forest lands in British Columbia. Fish. Oceans Can. Land Use Unit, Habitat Protection Division, Vancouver, B.C.

U.S. Environmental Protection Agency. 1975. Methods for acute toxicity tests with fish, macroinvertebrates and amphibians. Ecol. Res. Ser. EPA- 660/3-75-009. . Washington, D.C.

Van Leeuwen, C.J. and H. Maas. 1985. The aquatic toxicity of 2,6-dichlorobenzamide (BAM), a degradation product of the herbicide dichlobenil. Environ. Pollut. (Ser. A) 37 (2): 105-115.

Wan, M.T.K. 1986. The persistence of glyphosate and its metabolite amino-methyl-phosphonic acid in some coastal British Columbia streams. Environ. Can., Environ. Protection Serv. Regional Program Rep. No. 85-01. West Vancouver, B.C.

Wan, M.T.K, D.J. Moul, and R.G. Watts. 1987. Acute toxicity to juvenile Pacific salmonids of Garlon 3A, Garlon 4, triclopyr, triclopyr ester, and their transformation products: 3,5,6-trichloro-2-pyridinol and 2-methoxy-3,5,6-trichloropyridine. Bull. Environ. Contam. Toxicol. 39: 721-728.

Wan, M.T.K, R. Watts, and D. Moul. 1986. Toxicity to rainbow trout of selected formulations of 2,4-D and 2,4-D - diesel oil mixtures. Environ. Can., Environ. Protection Serv. Regional Program Rep. No. 85-02. West Vancouver, B.C.

___. 1987. Toxicity to salmonids of the herbicide glyphosate (Roundup®) and its surfactant (MON 0818). Regional Program Rep. No. 86-16. West Vancouver, B.C.

Wedemeyer, G.A., B.A. Barton, and D.J. McLeay. [1988]. Stress and acclimation. *In* Methods in fishery biology. P. Moyle and C. Schreck (editors). Am. Fish. Soc. Bethesda, Md. In press.

Wedemeyer, G.A., D.J. McLeay, and C.P. Goodyear. 1984. Assessing the tolerance of fish and fish populations to environmental stress: the problems and methods of monitoring. *In* Contaminant effects on fisheries. Vol. 16. V.W. Cairns, P.V. Hodson and J.O. Nriagu (editors). Advances in Environmental Science and Technology. J. Wiley & Sons, Toronto, Ont , pp. 163-195.

Wiersma-Roem, W.J., L.W.A. Vischer, E.M.H. Frederix-Wolters, E.G.M. Harmsen, P.D.A. Olthof, and O. deWilde. 1978. Sub-acute toxicity of the herbicide dichlobenil (2,6-dichloro- benzonitrile) in rainbow trout (*Salmo gairdnerii* R.) *In* Proc. EWRS 5th Symp. on Aquatic Weeds, Vol. 5., pp. 261-268.

Woodward, D.F. 1976. Toxicity of the herbicides dinoseb and picloram to cutthroat (*Salmo clarki*) and lake trout (*Salvelinus namaycush*). J. Fish. Res. Board Can. 33 (8): 1671-1676.

___. 1979. Assessing the hazard of picloram to cutthroat trout. J. Range Manage. 32 (3): 230-232.

Woodward, D.F. and F.L. Mayer, Jr. 1978. Toxicity of three herbicides (butyl, isooctyl, and propylene glycol butyl ether esters of 2,4-D) to cutthroat trout and lake trout. Tech. Pap. No. 97. U.S. Fish Wildl. Serv., U.S. Dep. Interior. Washington, D.C.

Yockim, R.S., A.R. Isensee, and E.A. Walker. 1980. Behavior of trifluralin in aquatic model ecosystems. Bull. Environ. Contam. Toxicol. 24: 134-141.

Yokote, M., S. Kimura, H. Kumada, and Y. Matida. 1976. Effects of some herbicides applied in the forest to the freshwater fishes and other aquatic organisms--IV. Experiments on the assessment of acute and subacute toxicities of 2,4,5-T to the rainbow trout. Bull. Freshwater Fish. Res. Lab. 26 (2): 85-93.

Zitko, V., D.W. McLeese, W.G. Carson, and H.E. Welch. 1976. Toxicity of alkyldinitrophenols to some aquatic organisms. Bull. Environ. Contam. Toxicol. 16 (5): 508-515.

APPENDIX 1. List of data bases searched

A. Computer Search

Key Words Searched

forestry (forest) herbicides
aquatic toxicity (lethal, sublethal)
bioassay(s)
bioassay protocol(s)
acute toxicity
chronic toxicity
salmonid fish (salmon, trout, char, grayling)
freshwater invertebrates (fishfood organisms)

Assisting Words Searched

1. *Chemicals of interest*

forest-use herbicides
pesticides (excluding insecticides)
brush control herbicides
conifer-release herbicides
aerial spray applications

amitrole	Roundup®	atrazine
2,4-D	glyphosate	Dicamba®
dinoseb	triclopyr	picloram
Silvex®	Garlon®	simazine
2,4,5-T	Krenite®	Velpar®
hexazinone	fosamine	

2. *Aquatic life of interest*

fisheries resource(s)
freshwater life, stream life, freshwater organisms
salmonidae, salmonid fish, salmonids
salmon (coho, chinook, pink, chum, sockeye, Atlantic, Pacific)
trout (rainbow, steelhead, brown, brook, speckled, lake, cutthroat, coastal cutthroat)
char, speckled char, dolly varden, Arctic grayling
life stage (egg, larvae, embryo, alevin, fry, fingerling, smolt, juvenile, adult)
daphnia, water fleas
chironomids, chironomid larvae, gammarids
mayfly, stonefly, midgefly
(salmonid) prey, food, food web, fish food, food chain

3. *Risk evaluation*

aquatic toxicity	laboratory (tests)	field (tests)
on-site bioassays	environmental (risk)	variables
environmental variables	exposure	duration of exposure
seasonal	recovery	bioassay(s)

toxicity test	test protocol	protocol(s)
bioassay method(s)	bioassay procedure	toxic effects
risk	threat	hazard
lethal toxicity	sublethal toxicity	acute toxicity
chronic toxicity	clearance	dispersal
dilution	flushing	degradation
conversion	death	mortality
behavioral	equilibrium	disequilibrium
physiological	biochemical	stress
smoltification	migration	tolerance
respiration	coloration	osmoregulation
reproduction	development	anomaly/deformity

Data Bases Searched

Aquaculture (1970-1987)
Aquaref (1970-1986)
Aquire
Chemical Abstracts (1969-1987)
Canadian Environment (1960-1987)
Chemical Exposure (1974-1987)
CRIS/USDA (1986-1987)
Enviroline (1971-1987)
Life Sci. Coll. (1978-1987)
OHM/TADS
Scisearch (1968-1987)
Zool. Record (1978-1986)
Aqualine (1960-1987)
Aquat. Sci. Abst. (1978-1987)
Biosis (1969-1987)
CAB (1972-1987)
CESARS
CODOC
ELIAS (1976-1987)
Environ. Bibliog. (1974-1987)
NTIS (1964-1987)
Poll Abst. (1970-1987)
Wat. Res. Abst. (1968-1987)

B. Manual Search

Periodicals Searched (1986/1987 Issues)

Aquat. Toxicol.
Bull. Environ. Contam. Toxicol.
Ecotoxicol. Environ. Safety
Environ. Sci. Technol.
Int. J. Environ. Studies
J. Environ. Qual.
J. Water Poll. Cont. Fed. (June)
ASTM STP Series
Arch. Environ. Contam. Toxicol.
Ecology
Environ. Pollut.
Hydrobiologia
J. Agric. Food Chem.
J. Range Manage.
Trans. Am. Fish. Soc.
CRC Series

APPENDIX 2. List of contacts

ASTM Committee E-47
Biological Effects & Environmental Fate
American Standards for Testing & Materials
1916 Race Street
Philadelphia, Pennsylvania 19103

Dr. Ian K. Birtwell
Fisheries Research Branch
Fisheries & Oceans Canada
West Vancouver Laboratory
4160 Marine Drive
West Vancouver, B.C. V7V 1N6

Dr. Kevin J. Buhl
U.S. Fish and Wildlife Service
National Fisheries Contaminant Research Center
Field Research Station
RR 1, Box 295
Yankton, South Dakota 57078

Dr. D.J. Call
Office of Toxic Substances
U.S. Environmental Protection Agency
Washington, D.C. 20460

Mr. H.T. Craven, Acting Chief
Ecological Effects Branch
Hazard Evaluation Division
U.S. Environmental Protection Agency
Washington, D.C. 20460

Mr. Dan Cronin
Pesticide Control Branch
Ministry of Environment & Parks
810 Blanshard Street
Victoria, B.C. V8V 1X5

Mr. Andrew Fabro
Conservation and Protection
Environment Canada
Kapilano 100, Park Royal
West Vancouver, B.C. V7T 1A2

Dr. Brian J. Finlayson
California Department of Fish & Game
Environmental Services Branch
Pesticide Investigations Unit
1701 Nimbus Road
Suite F
Rancho Cordova, California 95670

Dr. L.C. Folmar
U.S. Fish and Wildlife Service
Fish-Pesticide Research Unit
Denver Federation Center
Denver, Colorado 80225

Director, Forest Pest Management Institute
Canadian Forestry Service
Department of the Environment
P.O. Box 490
Sault Ste. Marie, Ontario P6A 5M7

Dr. W.A. Hartman
Columbia National Fisheries Research Laboratory
Field Research Station
Yankton, South Dakota 57078

Mr. John Henigman
Pest Management
Protection Branch
Ministry of Forests
31 Bastion Square
Victoria, B.C. V8W 3E7

Mr. Steve Horvath
Environmental Laboratory
Ministry of Environment and Parks
3650 Wesbrook Mall
Vancouver, B.C. V6S 2L2

Dr. A.R. Isensee
U.S. Environmental Protection Agency
Pesticide Degradation Laboratory
Beltsville, Maryland 20705

Dr. N.Y. Khan
Pesticides Evaluation Officer
Fish Habitat Management Branch
Fisheries and Oceans Canada
Ottawa, Ontario K1A 0E6

Dr. Peter Kingsbury
Project Leader, Environmental Impact
Forest Pest Management Institute
Canadian Forestry Service
P.O. Box 490
Sault Ste. Marie, Ontario P6A 5M7

Mr. J.H. Little
Director, Protection Branch
Ministry of Forests
31 Bastion Square
Victoria, B.C. V8W 3E7

Dr. Harry Lorz
Oregon Department of Fish and Wildlife
Research and Development Section
303 Ballard Extension Hall, O.S.U.
Corvallis, Oregon 97331

Dr. Jim Malick
Norecol Environmental Consultants Ltd.
600 - 1281 West Georgia Street
Vancouver, B.C. V6E 3J7

Dr. Monte A. Mayes
Environmental Sciences Research
Dow Chemical U.S.A.
Midland, Michigan 48640

Dr. Michael Newton
Department of Forest Science
Oregon State University
Corvallis, Oregon 97331

Dr. Logan N. Norris
U.S. Department of Agriculture, Forest Service
Pacific Northwest Forest & Range
Experiment Station
Forestry Science Laboratory
Corvallis, Oregon 97331

Office of Pesticides
U.S. Environmental Protection Agency
401 M Street SW
Washington, D.C. 20460

Office of Toxic Substances
U.S. Environmental Protection Agency
401 M Street SW
Washington, D.C. 20460

Dr. D. Palawski
Columbia National Fisheries Research Laboratory
U.S. Department of the Interior
U.S. Fish and Wildlife Service
Route 1
Columbia, Missouri 65201

Dr. Roberta Parish
Program Manager Assistant
Northern Interior TAC
Research Branch
Ministry of Forests
31 Bastion Square
Victoria, B.C. V8W 3E7

Mr. D. Poirier
Invertebrate Toxicologist
Aquatic Biology Section
Water Resources Branch
Ontario Ministry of the Environment
P.O. Box 213
Rexdale, Ontario M9W 5L1

Mr. Steve Samis
Water Quality Unit
Habitat Management Division
Fisheries & Oceans Canada
400 - 555 West Hastings Street
Vancouver, B.C. V6B 5G3

Dr. H.O. Sanders
U.S. Department of the Interior
Fish and Wildlife Service
Columbia National Fisheries Research Laboratory
Columbia, Missouri 65201

Mr. Rick Scroggins
Environment Canada
351 St. Joseph Boulevard
Thirteenth Floor
Hull, Quebec K1A 0H3

Mr. Gary A. Sergy
Technology Development & Technical
Services Branch
Environmental Protection, Conservation
and Protection Environment Canada
Twin Atria #2, 4999 - 98th Avenue
Edmonton, Alberta T6B 2X3

Dr. James A. Servizi
Cultus Lake Salmon Research Laboratory
Biological Sciences Branch
Fisheries & Oceans Canada
Cultus Lake, B.C. V0X 1H0

Dr. M. Sharom
Environmental Affairs Specialist
Agricultural Products
Du Pont Canada Inc.
Box 2300, Streetsville
Mississauga, Ontario L5M 2J4

Mr. M.P. Stewart
Pesticides Directorate
Agriculture Canada
S.B.I. Building
2nd Floor
2323 Riverside Drive
Ottawa, Ontario K1A 0C6

Mr. Al Switzer
Senior Development Specialist
Dow Chemical Canada Inc.
Suite 2412
10025 Jasper Avenue
Edmonton, Alberta T5J 1S6

Mr. Larry Taylor
Regional Technical Representative
Monsanto Canada Inc.
Delta, B.C.

Mr. Phil Thomas
Microbics Enterprises
RR #2
Forest, Ontario N0N 1J0

Mr. Ray Travers
Recreational Fisheries Branch
Ministry of Environment & Parks
780 Blanshard Street
Victoria, B.C. V8V 1X5

U.S. Department of Agriculture
Forest Service
Pacific Northwest Forest & Range
Experimental Station
Portland, Oregon

Ms. Sylvia von Schuckmann
Recreational Fisheries Branch
Ministry of Environment & Parks
780 Blanshard Street
Victoria, B.C. V8V 1X5

Mr. Mike Wan
Conservation and Protection
Environment Canada
Kapilano 100, Park Royal
West Vancouver, B.C. V7T 1A2

Mr. Ron Watts
Aquatic Toxicity Laboratory
Conservation and Protection
Environment Canada
1801 Welch Street
North Vancouver, B.C. V7P 1B7

Dr. Michael Wong
Water Quality Branch
Inland Waters/Lands
Environment Canada
Ottawa, Ontario K1A 0H3

Dr. D.F. Woodward
U.S. Fish & Wildlife Service
Field Research Laboratory
Jackson, Wyoming 83001